The effect of oxygen stress (hypoxia and anoxia) on free-living marine nematodes in coastal ecosystems

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مهرشاد طاهری

سی یکم خرداد هزاروسیصد ونود وپنج

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Summary

Over the last century, hypoxia (dissolved oxygen < 63 µmol l^{-1} or 2 mg l^{-1}) and anoxia (dissolved oxygen = 0 µmol l^{-1}) in coastal ecosystems has increased due to eutrophication and climate change (Diaz and Rosenberg, 2008; Stramma et al., 2008). This oxygen deficiency does not only affect biogeochemical processes in the sediment (Middelburg and Levin, 2009) but it also adversely affects the benthic faunal life (Riedel et al., 2014). Responses of marine benthic communities (macro- and meiofauna) to oxygen stress depend on the duration and intensity of stress (Modig and Olafsson, 1998; Levin et al., 2009). In general, macrofauna are more sensitive than meiofauna to oxygen stress (Levin et al., 2009). Among the meiofauna, copepods are the most sensitive, and Foraminifera are the most resistant group to oxygen stress in coastal sediments (Grego et al., 2014; Langlet et al., 2014). Marine nematodes are resistant to short-term oxygen stress but sensitive to longer periods of exposure. Marine free-living nematodes are the most abundant metazoans in sediments (Giere, 2009) and play an important role in ecological processes such as nutrient cycling, organic matter mineralization, and micro bioturbation of sediments (Nascimento et al., 2012; Moens et al., 2013; Bonaglia et al., 2014).

A recent study showed that rates in oxygen decline are more severe in the coastal areas than in the open oceans (Gilbert et al., 2010). In this PhD research, the responses of nematode community characteristics from different sediment types and geographical locations to oxygen depletion (hypoxia and anoxia) were studied. We also tested whether short-term hypoxia affected nematode feeding activity. In addition, the effects of both short- and long-term anoxia, and the recovery of the nematode

communities after long periods of anoxia were investigated for the first time in a single field experiment.

Chapter 1 provides a general overview of the distribution of oxygen in the ocean, coastal ecosystem, the reasons of oxygen stress (ocean warming and eutrophication), along with the effect of oxygen depletion on marine benthic biodiversity and nematodes. Objectives, study areas and outline of the thesis are described.

We assumed that nematode communities living naturally in well oxygenated (coarser) sediments were less tolerant to oxygen stress than communities from finer sediment. Therefore, in Chapter 2, the responses of nematode communities to short-term hypoxia (1 and 7 days) were studied in three North Sea stations with contrasting sediment types (coarse silt, fine sand and medium sand). In the field, nematode density, diversity, vertical distribution and community structure differ among the stations. After subjecting the nematode communities to 1 and 7 days of hypoxia in the lab, we found no effect on total density, diversity, community structure, vertical density profiles (except in the fine sand) and densities of five dominant species in all sediment types. Therefore, we accept the null hypothesis (H0) that duration of hypoxia does not affect the nematode community characteristics in the different sediment types.

The negative effect of anoxia on nematode community is related to its duration (Moodley et al., 1997). In Chapter 3, the effect of both short and long-term artificially induced anoxia on a benthic nematode community, and its potential for recovery after natural reoxygenation were investigated in an *in situ* experiment on a silty-sand bottom in the Gulf of Trieste (northern Adriatic Sea). Anoxia was created by deploying three underwater benthic Plexiglas chambers (50×50×50 cm) at a depth of 24 meter.

Treatments lasted for 2, 23 and 307 days. After opening the chambers, samples were obtained after 7, 30 and 90 days to investigate the recolonization and recovery patterns of the nematode communities.

Our results revealed that short-term anoxia (2 days) did not affect nematode total density and diversity, community structure and their vertical distribution in the silty-sand sediment, which confirms our first experiment (Chapter 2). However, total and vertical nematode density, species richness and diversity decreased after 23 days of anoxia, and decreased even further after 307 days of anoxia. 21 nematode species among which *Metalinhomoeus effilatus, Paralinhomoeus caxinus* and *Terschellingia longicaudata* even survived at 307 days anoxia treatment. Our findings also demonstrated that nematode communities exposed to 23 days of anoxia did not recover (total density and diversity indices) after 30 days sediment reoxygenation while a full recovery of the nematode community characteristics was observed after 90 days for sediment exposed to 307 days of anoxia.

Feeding type contribution (functional aspect) of the nematode community also changed as a consequence of anoxia, and during the recolonization and recovery processes. This change was most drastic after 23 and 307 days of Anoxia. At both normoxia and 2 days of anoxia, selective deposit feeders (1A), non-selective deposit feeders (1B) and epistrate feeders (2A) nematodes were observed among the dominant nematode community. Epistrate feeders disappeared from the 23 days of anoxia treatment and from the 307 days of anoxia, while non-selective deposit feeders (1B) were the only important feeding type remaining in the nematode community. After the recolonization and recovery process, epistrate feeders and selective deposit feeders reappeared again

amongst the dominant nematode species after 30 and 90 days of recovery, respectively. Therefore, we reject both null hypotheses (H0) i) the duration of *in situ* anoxia does not affect the nematode community characteristics (density, diversity and vertical distribution, and ii) the recovery of the nematode community is not related to the duration of anoxia.

Our results from Chapters 2 and 3 showed a general capacity of nematode communities to survive short-term oxygen stress. A decreased respiration rate (metabolism) (Braeckman et al., 2013), together with the ability to switch between aerobic and anaerobic metabolism (Tahseen, 2012) has been reported as physiological adaptations of nematodes to tackle oxygen stress. As some part of the obtained oxygen is generally used for complete oxidation of food in aerobic metabolism pathways, we investigated whether a decreasing nematode feeding activity is a strategy to build up resistance to short term-hypoxic condition. Therefore, in Chapter 4, the effect of short-term hypoxia (6 days) on the feeding activity of dominant nematode genera from an intertidal mudflat (Paulina, Westerschelde, south-west Netherlands) was studied in a laboratory experiment. Nematode samples were taken from the Paulina intertidal flat in the Westerschelde estuary, cores were transferred to the lab (16 °C) where oxic and hypoxic conditions were created by bubbling the overlying water with air and N_2. The effect of hypoxia on the feeding activity of nematodes was measured by adding ^{13}C pre-labelled diatoms at the sediment-water interface in Oxic and Hypoxic treatments, and following the uptake of the labelld diatoms (*Cylindrotheca fusiformis*) in dominant nematode genera after 6 days. Results revealed a low carbon uptake by all genera suggesting that the added diatoms only represented a limited food source for the

studied nematode genera. However based on our results, there was no significant decrease in the feeding activity of all dominant nematode genera in the Hypoxic treatments. Hence, we accept the null hypothesis (H0) that short-term hypoxia (6 days) does not affect the feeding activity of nematode communities.

The acquired knowledge on the effect of severity and duration of oxygen stress on marine coastal nematode communities is summarized and integrated in Chapter 5. In conclusion, coastal nematode communities are tolerant to short-term oxygen stress (<7 days) in term of density, species diversity, community structure, vertical distribution and feeding activity. In contrast, longer term anoxia has a negative impact on the coastal nematode community, and causes changes in the relative contributions of the different feeding types among the dominant nematode species. While nematode density and diversity decreased during a long-term exposure to anoxia, some nematode species survived, which could result in the maintenance of some important benthic processes which are performed by these tolerant nematodes (i.e. nutrient cycling). In areas where hypoxia/anoxia occurs regularly, it is possible that the nematode community structure shifts to a community dominated by a limited number of tolerant species occurring in high densities over a long period of time as observed in many coastal ecosystems worldwide (Rabalais et al., 2001; Baustian and Rabalais, 2009).

Samenvatting

Tijdens de laatste eeuw werden kustecosystemen in toenemende mate getroffen door hypoxia (opgeloste zuurstof < 63 µmol l^{-1} or 2 mg l^{-1}) en anoxia (opgeloste zuurstof = 0 µmol l^{-1}) als gevolg van eutrofiëring en klimaatsverandering (Diaz and Rosenberg; 2008, Stramma et al., 2008). Dit heeft niet alleen een effect op de biogeochemische processen in het sediment (Middelburg and Levin, 2009), ook negatieve effecten op het bodemleven werden aangetoond (Riedel et al., 2014 voor een overzicht). De respons van mariene bodembewonende gemeenschappen (meio- en macrobenthos) is afhankelijk van de duur en de intensiteit van de zuurstofstress (Modig en Olafsson, 1988; Levin et al., 2009). Het macrobenthos blijkt gevoeliger te zijn voor zuurstofstress dan de meiobenthische gemeenschappen, roeipootkreeftjes (Copepoda) worden als heel gevoelig beschouwd en Foraminifera zijn gekend als tolerante leden van het meiobenthos in sedimenten van kustzeeën (Grego et al., 2014; Langlet et al., 2014). Vele studies hebben verder aangetoond dat vrijlevende nematoden tolerant zijn aan zuurstofstress van korte duur, maar bij langere periodes van lage zuurstofconcentraties zullen alleen enkele tolerante soorten kunnen overleven. Mariene vrijlevende nematoden vormen de meest abundante groep binnen het meiobenthos. Door hun hoge abundanties spelen ze een belangrijke rol in ecologische processen zoals de nutriëntencycli, de mineralisatie van organisch materiaal, en microbioturbatie van sedimenten (Nascimento et al., 2012; Moens et al., 2013; Bonaglia et al., 2014).

Uit recent onderzoek blijkt dat de zuurstofproblematiek sterker aanwezig is in de kustzone dan in de open oceanen (Gilbert et al., 2010). In voorliggend proefschrift wordt de respons van de structurele kenmerken van nematodengemeenschappen, afkomstig

uit verschillende sedimenten uit verschillende geografische lokaties, op zuurstofstress (hypoxie en anoxie) bestudeerd. bestudeerd. Daarnaast wordt nagegaan of de voedingsactiviteit van nematodengemeenschappen beïnvloed wordt door korte periodes van verminderde zuurstofconcentratie. Als laatste punt wordt zowel de respons als het herstel van nematodengemeenschappen die blootgesteld worden aan langere periodies van zuurstofstress onderzocht in een veldexperiment.

In hoofdstuk 1 wordt een algemeen overzicht gegeven van de verspreiding van zuurstof in oceanen en kustecosystemen, en worden de oorzaken van zuurstofstress (opwarming van de oceanen en eutrofiëring) belicht. Daarnaast wordt dieper ingegaan op het effect van verlaagde zuurstofconcentraties op mariene biodiversiteit en nematogemeenschappen. De verschillende doelstellingen en studiegebieden voor dit onderzoek worden voorgesteld, en de opbouw van dit proefschrift wordt toegelicht.

Er wordt verondersteld dat nematodengemeenschappen, die afkomstig zijn uit (grovere) sedimenten die goed voorzien zijn van zuurstof, minder goed aangepast zijn aan zuurstofstress dan soorten nematoden die van nature in fijnere sedimenten leven. Daarom wordt in hoofdstuk 2 gerapporteerd over een experiment waarbij nematodengemeenschappen afkomstig uit verschillende sedimenten (slibbig zand, fijn zand en medium zand) worden blootgesteld aan hypoxia gedurende 1 en 7 dagen. Er worden geen effecten waargenomen op de aantallen, diversiteit, gemeenschapsamenstelling en vertikale verdeling van de nematoden in de bodem. Daarom aanvaarden we de nulhypothese dat de termijn van blootstelling aan hypoxia geen invloed heeft op de gemeenschapskenmerken van nematodengemeenschappen uit verschillende sedimenten.

Moodley et al. (1997) hebben aangetoond dat het effect van anoxia op nematodengemeenschappen afhankelijk is van de duur van de zuurstofstress. Daarom werd in hoofdstuk 3 onderzocht hoe nematodengemeenschappen kunnen veranderen (i.e. zich kunnen aanpassen) als gevolg van zuurstofstress. In de Golf van Trieste (Adriatische Zee) werd een veldexperiment uitgevoerd, waarbij op kunstmatige wijze anoxia werd geïnduceerd voor korte en langere periodes. Daarnaast werd ook nagegaan hoe het herstel van nematodengemeenschappen verloopt na verschillende periodes van anoxia. De anoxische omgeving werd gecreëerd door middel van benthische kamers (50 x 50 x 50 cm) die op de bodem bleven gedurende respectievelijk 2, 23 en 307 dagen. Na het openen van de kamers, werd het herstel van de gemeenschappen opgevolgd door het nemen van stalen na 7 dagen (Anoxia 2D), 30 dagen (Anoxia 23D) en 90 dagen (Anoxia 307D). De resultaten tonen aan dat densiteit, diversiteit, samensteling en vertikale verdeling van nematodengemeenschappen niet veranderen na 2 dagen anoxia, wat de resultaten uit het vorige hoofdstuk bevestigt. Na 23 dagen nemen totale densiteit en densiteit per sedimentlaag, soortenrijkdom en diversiteit af. Deze tendens zet zich verder na 307 dagen anoxia. 21 nematodensoorten overleefden het experiment na 307 dagen, waaronder *Metalinhomoeus effilatus, Paralinhomoeus caxinus* en *Terschellingia longicaudata*. Het experiment toont verder aan dat er geen volledig herstel optrad na 30 dagen van hernieuwde zuurstoftoevoer, voor nematodengemeenschappen die 23 dagen werden blootgesteld aan anoxische omstandigheden. Bij de nematodengemeenschappen die 307 dagen in anoxische omstandigheden doorbrachten, wordt wel een volledig herstel waargenomen, 90 dagen na het openen van de experimentele eenheden.

De samenstelling volgens voedingstype (een functioneel aspect) van de nematodengemeenschappen veranderde bij blootstelling aan anoxia, en verder tijdens de rekolonisatie- en herstelperiode. De sterkste veranderingen werden waargenomen bij de Anoxia 23D en 307D behandelingen. Onder normoxische omstandigheden, en in de Anoxia 2D behandelingen waren selectieve en niet-selectieve depositvoeders (1A en 1B), epistratum (diatomeeën) eters (2A) vertegenwoordigd bij de dominante soorten van de nematodengemeenschappen. De 2A-nematoden verdwenen uit de gemeenschappen die werden aangetroffen in het Anoxia 23D experiment, terwijl de 1B-nematoden de enige belangrijke vertegenwoordigers waren na 307 dagen anoxia. Na de rekolonisatie- en herstelperiode waren de epistratum-voeders en de selectieve depositvoeders opnieuw aanwezig onder de dominante soorten na een periode van respectievelijk 30 en 90 dagen. Daarom werden de nulhypotheses (1) “de duur van zuurstofstress heeft geen invloed op de densiteit, diversiteit en vertikale verdeling van de nematodengemeenschappen” en (2) het herstel van nematodengemeenschappen is onafhankelijk van de duur van de anoxische periode” verworpen.

De resultaten uit hoofdstukken 2 en 3 tonen aan dat nematodengemeenschappen korte periodes van zuurstofstress kunnen overleven. Er werd reeds gemeld dat een verminderde respiratiesnelheid (Braeckman et al., 2013), samen met de mogelijkheid om om te schakelen naar een anaerobe stofwisseling (Tahseen, 2012), kunnen worden aanzien als fysiologische aanpassingen om zuurstofstress te weerstaan. Omdat een deel van de zuurstof die wordt opgenomen door nematoden wordt gebruikt voor aerobe stofwisseling, gingen we na of een verminderde voedingsactiviteit kan worden waargenomen onder hypoxische omstandighen van korte duur. In hoofdstuk 4 wordt

gerapporteerd over een experiment waarbij de voedingsactiviteit van nematoden uit een intertidale zandplaat (Paulina, Westerschelde, ZW Nederland) werd opgevolgd na 6 dagen hypoxia. Sediment werd verzameld op Paulina, en vervoerd naar het labo (16°C) waar oxische en hypoxische omstandigheden werden gecreëerd door het bovenstaand water te bubbelen met lucht en N_2. Aan de experimentele opstellingen werden diatomeeën (*Cylindrotheca fusiformis*) toegevoegd die vooraf gelabeld waren met ^{13}C. De opname van label door de dominante nematodengenera werd gemeten na 6 dagen experimentele omstandigheden. Onze resultaten toonden een algemene lage opname van koolstof door alle nematodengenera. Dit kan te wijten zijn aan het feit dat de toegevoegde diatomeeën slechts een beperkte voedselbron zijn voor de onderzochte genera. Onze resultaten duiden verder geen significante afname van voedingsactiviteit door door nematoden in hypoxische omstandigheden. Daarom wordt de nulhypothese dat korte periodes van hypoxia geen effect hebben op de voedingsactiviteit van nematode aanvaard.

In hoofdstuk 5 wordt de nieuwe kennis omtrent het effect van intensiteit en duur van zuurstofstress op nematodengemeenschap samengevat en geïntegreerd. Als besluit kan gesteld worden dat nematodengemeenschappen uit kustecosystemen goed bestand zijn tegen korte periods (minder dan 7 dagen) van zuurstofstress, voor wat betreft densiteit, soortendiversiteit, gemeenschapsamenstelling, vertikale verdeling en voedingsactiviteit. Langere periodes van anoxia resulteren echter wel in negatieve effecten die gepaard gaan met veranderingen in de relatieve aantallen van de verschillende voedingstypes onder de dominante soorten. Tijden deze lange periodes van zuurstofloosheid nemen de aantallen en diversiteit af. Echter, sommige

nematodensoorten kunnen deze periode overleven, wat zou kunnen resulteren in het behoud van een aantal belangrijke benthische ecosysteemprocessen (vb. nutriëntenkringloop) die door deze tolerante soorten worden gefaciliteerd.

In gebieden waar hypoxia/anoxia zich op regelmatige basis voordoet, bestaat de mogelijkheid dat de samenstelling van de nematodengemeenschappen verschuift naar een gemeenschap die gedomineerd wordt door een beperkt aantal tolerante soorten, die dan voor langere periodes in hoge aantallen voorkomen, zoals werelwijd gerapporteerd voor vele kustecosysyememen (Rabalais et al., 2001; Baustian and Rabalais, 2009).

Chapter 1. General introduction, aims and thesis outline

1. The distribution of oxygen in the ocean

The oxygen exchanges across the air-sea surface and oxygen produced by phytoplankton and other aquatic plants are the most important sources of oxygen in the marine environment. It is estimated that 70% of the world's oxygen is produced via photosynthesis by phytoplankton (Pal and Choudhury, 2014). Oxygen is not only a vital element for all aerobic animals but it also plays an important role in the chemical cycling of carbon, nitrogen and many other important elements like iron and manganese, both in the water and within the sediment (Kristiansen et al., 2002; Keeling et al., 2010; Metzger et al., 2014). The concentration and distribution of the dissolved oxygen in the water column is a result of the interplay between oxygen production and consumption, and is largely affected by various factors including light availability, nutrient concentrations, salinity, temperature, atmospheric exchange, upwelling and physical transport (Garcia and Gordon, 1992; Los et al., 2008; Peña et al., 2010). Generally, the surface water has the maximum oxygen concentration due to atmospheric exchange and oxygen release by photosynthesis processes. In the water column, oxygen is consumed by respiration, mineralization of organic matter, nitrification and redox reactions (Peña et al., 2010) and also declines due to ocean warming (Keeling et al., 2010; Moffitt et al., 2015). In the sediment, oxygen dynamics are related to bottom water oxygen concentrations, diffusive boundary layers, faunal activity and consumption, ocean warming, mineralization, hydrodynamic forces and sediment permeability (Fig. 1, Wu, 2002; Glud, 2008; Huettel et al., 2014).

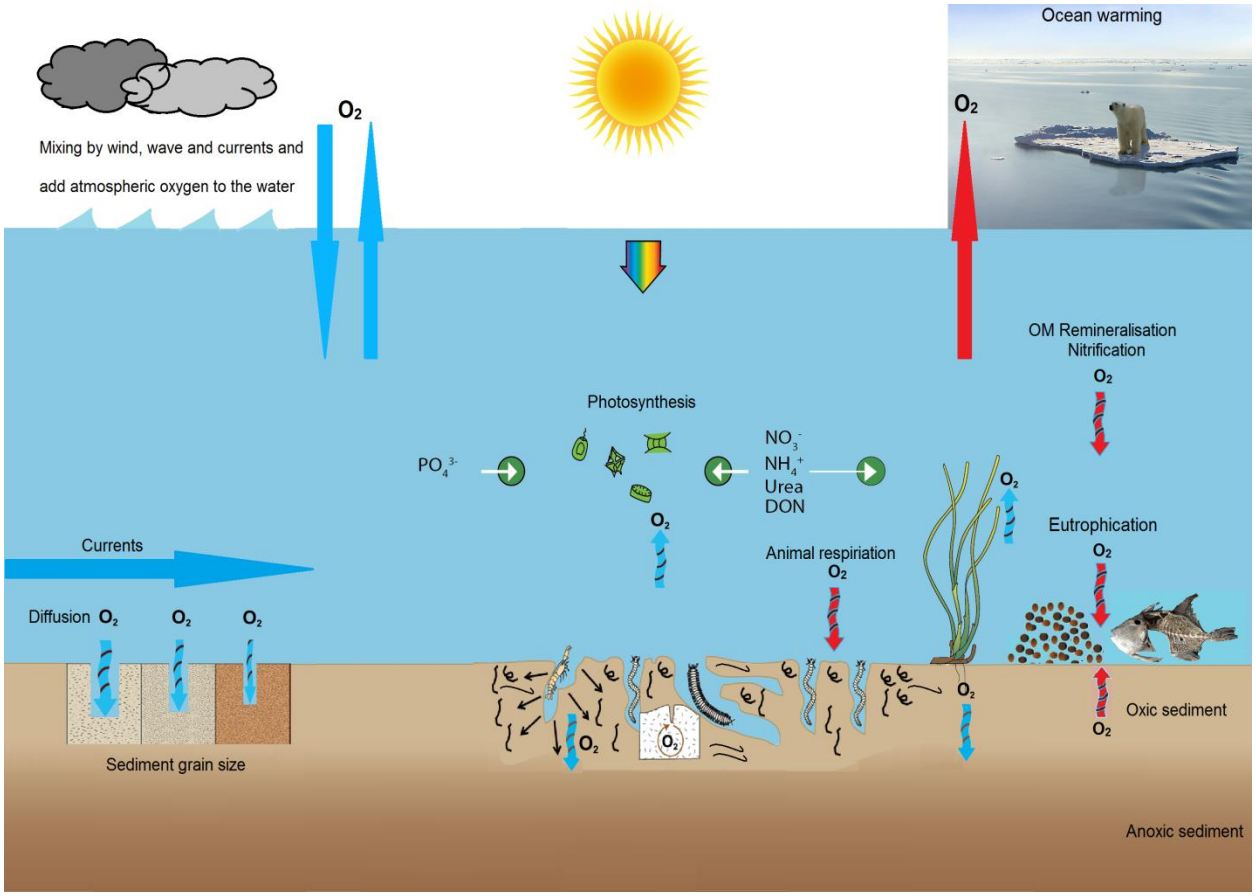


Figure 1. Factors affecting the vertical distribution and consumption of the dissolved oxygen in the water column, sediment and their relationships. OM = organic matter (modified after: http://www.water.wa.gov.au/water-topics/rivers-and-estuaries/assessing-waterway-health/estuary-sediment-quality and Peña et al., 2010).

Hypoxia (dissolved oxygen < 63 µmol l^{-1} or 2 mg l^{-1}) and anoxia (dissolved oxygen = 0 µmol l^{-1}) can occur naturally in some marine ecosystems, depending on the local topography, natural inputs of the organic matter, water circulation and temperature (Levin et al., 2009; Rabalais et al., 2010). The Black Sea, Baltic Sea and fjords (e.g. Gullmar fjord, west coast of Sweden) with long water residence time and little exchange with sources of oxygenated water are the best examples of such impacted ecosystems (Levin et al., 2009).

Oxygen depletion in coastal ecosystems has increased directly or indirectly by human activities (Fig. 2, Diaz and Rosenberg, 2008; Vaquer-Sunyer and Duarte, 2008). Hypoxia in the northern Gulf of Mexico is a good example of the effect of human activities (Wetzel et al., 2001; Levin et al., 2009). It is adjacent to, and influenced by the Mississippi and Atchafalaya Rivers. High riverine inputs of nutrients resulted in eutrophication and the development of hypoxia. The maximum hypoxic area at depths between 5 and 45 m was 13500 km^2 in 1985 and reached to 22000 km^2 in 2002 (Rabalais et al., 2007).

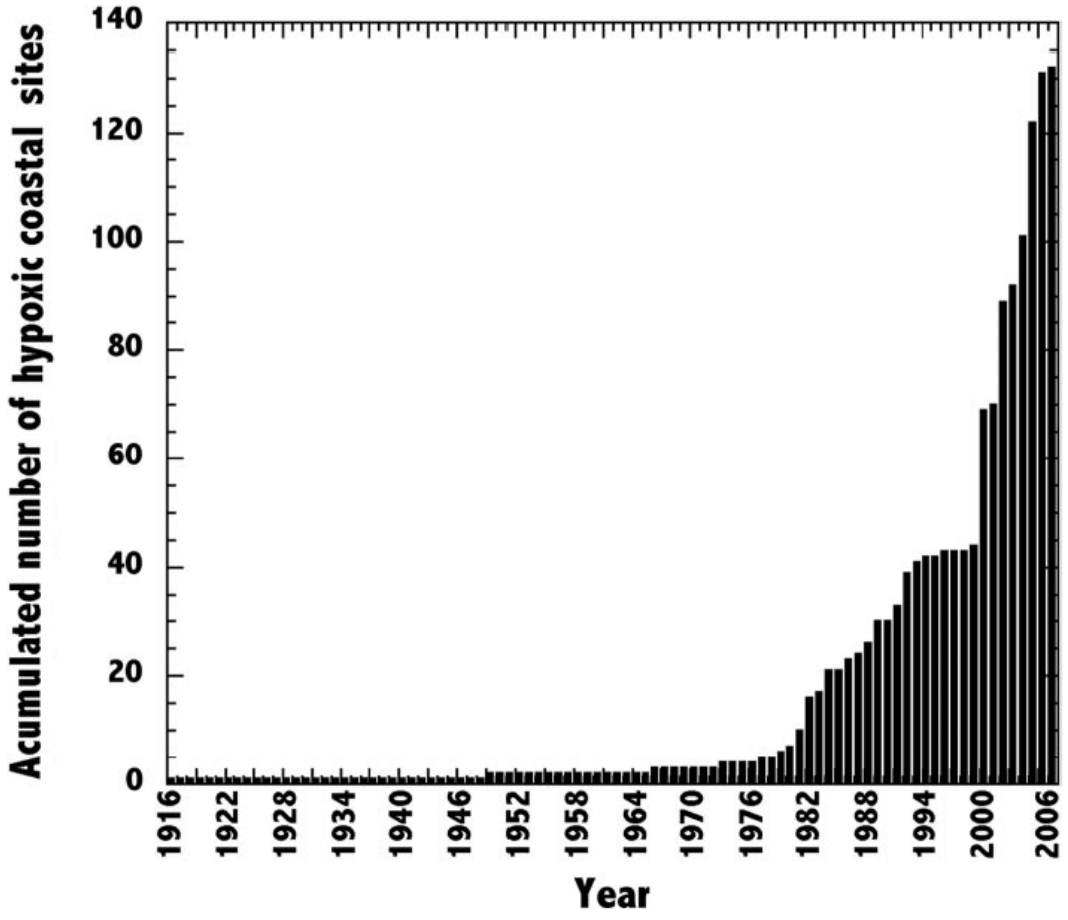


Figure 2: An increase in the number of coastal sites where hypoxia has been reported during last century (Vaquer-Sunyer and Duarte, 2008).

2. Coastal ecosystems under anthropogenic stress

Coastal ecosystems are at the boundary between the oceans, the atmosphere and the terrestrial biosphere, and underline their importance for instance in environmental processes such as exchange of gases (O_2 and CO_2) between atmosphere and water, climate regulation and nutrient cycling (Boaden and Seed, 1996; Costanza, 1999; McLean et al., 2001). The diversity of coastal ecosystems, including estuaries, wetlands, mangroves, sea grass beds, coral reefs and kelp forests renders the coastal zones among the biologically most diverse parts of the oceans (e.g. Scavia et al., 2002; Keller and Causey, 2005). The coastal environments cover around 10% of the global ocean area, where over 20% of the global marine primary production occurs (Wollast, 1998).

The marine coastal areas (water depth < 200 m) play an important role in human life (Crowell et al., 2010; Neumann et al., 2015). The relative ecological and economical importance of the coastal zones is very high compared with the open oceans (Pauly and Christensen, 1995; Costanza et al., 1997; Costanza, 1999), especially their role in global transportation, fisheries and tourism industries are of paramount importance (Costanza, 1999). Human populations in the coastal areas are growing fast, resulting in more industrialisation and agricultural activity (Neumann et al., 2015) causing environmental degradation (e.g. Magni, 2003; Rabalais et al., 2009).

The main impacts of human activities on coastal waters are:

1- Biological invasions (e.g. Stachowicz et al., 2002; Roohi et al., 2010)

2- Overfishing (e.g. Jackson et al., 2001)

3- Habitat destruction (e.g. Jackson, 2008)

4- Coastal pollutions and degradation of water quality (e.g. Smith et al., 1999; Derraik, 2002)
5- Ocean acidification (e.g. Guinotte and Fabry, 2008; Jackson, 2008)
6- Ocean warming (e.g. Harley et al,, 2006; Boesch, 2008)
7- Coastal eutrophication (e.g. Smith et al., 1999; Rabalais et al., 2009)

Especially ocean warming and coastal eutrophications, and their combined effects, are linked to oxygen depletion in the bottom waters of the shallow coastal areas (Diaz and Rosenberg, 2008; Vaquer-Sunyer and Duarte, 2008; Conley et al., 2009; Meire et al., 2013). The duration of oxygen depletion (hypoxia and anoxia) is different in different ecosystems, and related to local topography, currents, organic matter and nutrient loadings and timing and magnitude of the phytoplankton bloom (Levin et al., 2009). The oxygen stress may last hours (D' Avanzo and Kremer, 1994), days to weeks (Norkko and Bonsdorff, 1996) and even months to years (Jørgensen, 1980). Recent studies indicate greater oxygen decline rates in the coastal areas than in the open oceans as a result of climate change and coastal eutrophication (e.g. Gilbert et al., 2010).

3. Relation between ocean acidification and oxygen stress

Ocean acidification is the decline in the pH of the Earth's oceans, caused by the uptake of carbon dioxide (CO_2) from the atmosphere (Caldeira and Wickett, 2003). It is estimated that 30–40% of the anthropogenic carbon dioxide released into the atmosphere, dissolves into oceans and other aquatic ecosystems (Millero, 1995; Feely et al., 2004). An increase in carbon dioxide partial pressure (pCO_2) in surface waters by 2–3 folds is predicted by the end of the century (Melzner et al., 2013) which let to a 0.5

unite decline in the global mean surface pH by 2100 (Caldeira and Wickett, 2003; Caldeira, 2005). There is also a relationship between oxygen stress and ocean acidification. Recent studies showed that low dissolved oxygen and low pH conditions co-occur in many coastal and open ocean environments (e.g. Melzner et al., 2013; Mora et al., 2013; Gobler and Baumann, 2016) but their synergistic effects on macrobenthic fauna are rarely explored (e.g. Gobler et al., 2014; Steckbauer et al., 2015). For example, Gobler et al. (2014) reported a reduction in growth of two marine bivalvs as a result of the combined exposure to low oxygen and low pH. Decreases in respiration rate in different marine organisems as a result of these combine effect were also reported (Steckbauer et al., 2015). The concurrent effects of low dissolved oxygen and low pH on marine meiofauna are are largely unknown. Although morphological changes could be an adaptation in Foraminifera to cope with oxygen stress, these changes make them more vulnerable to dissolution in the low pH conditions, especially in OMZs (Zeppilli et al., 2015). It is shown that exposure to acidified seawater could significantly alter community structure and reduce the diversity of nematode assemblages (Widdicombe et al., 2009). The relative effect of these stressors (oxygen depletion and low pH) as well as the threshold levels for change and when secondary stressors become important are still unclear for marine organisms (Gobler et al., 2014).

4. The effect of ocean warming on oxygen depletion

Global warming is a term referring to the gradual increase of average temperature of the Earth's climate system (about 0.8°C) since 1880, and has important effects on different ecosystems (Ring et al., 2012). As a consequence of global warming, the global ocean

surface water has warmed up substantially over the past 50 years (Stramma et al., 2008), with rates of increase of 0.13 ºC per decade during the last 30 years (Casey and Cornillon, 2001). This can induce shifts in oceans pH, oxygen concentration, and productivity, which in turn could alter biological and social systems (Mora et al., 2013). Ocean warming is predicted to decline oceanic oxygen concentrations between 1 to 7 percent of the global ocean oxygen inventory by the year 2100 (Keeling et al., 2010; Moffitt et al., 2015). Furthermore, higher temperature enhances respiratory oxygen demand of animals and increases oxygen consumption rates in sediments (Wu, 2002). In addition, increasing temperature may create stronger ocean stratification, thereby reducing the oxygen transfer to the deeper waters (under the thermocline) and the seafloor ecosystem (Keeling and Garcia, 2002; Conley et al., 2009; Peña et al., 2010). When the deeper water is stagnating, the oxygen concentration will rapidly decrease due to consumption by animals or mineralization processes (Middelburg et al., 1993; Feely et al., 2004; Whitney et al., 2007; Fig. 3). Hypoxia driven by ocean warming and stratification can have a negative effect on marine benthic communities in coastal waters. In the Mariager Fjord (Denmark), high temperatures in summer increased the rate of oxygen consumption by animals and the lack of wind mixing limited ventilation of deeper waters. As a consequence, mussels experienced mass mortality and mineralization of their dead bodies together with an increased oxygen demand due to the decomposition of phytoplankton, resulted in oxygen depletion (Rabalais et al., 2010).

The effect of ocean warming in not restricted to the coastal waters. Low oxygen zones also develop within deeper waters (more than 100 m) of tropical and temperate oceans

(Gobler and Baumann, 2016). A decline in the oxygen concentration has been reported from depths of 100 to 400 m in the subarctic Pacific from 1956 as a consequence of ocean warming (Whitney et al., 2007). The decrease in oxygen concentration in the bottom water (300 – 700 m) has also been reported in the eastern tropical Atlantic and the equatorial Pacific during the past 50 years by global warming (Stramma et al., 2008). This can reduce the density of benthic fauna (mega-macro and meiofauna) as a consequence, following a reduction in bioturbation and changes in nutrient cycles in Oxygen Minimum Zone (Levin, 2003).

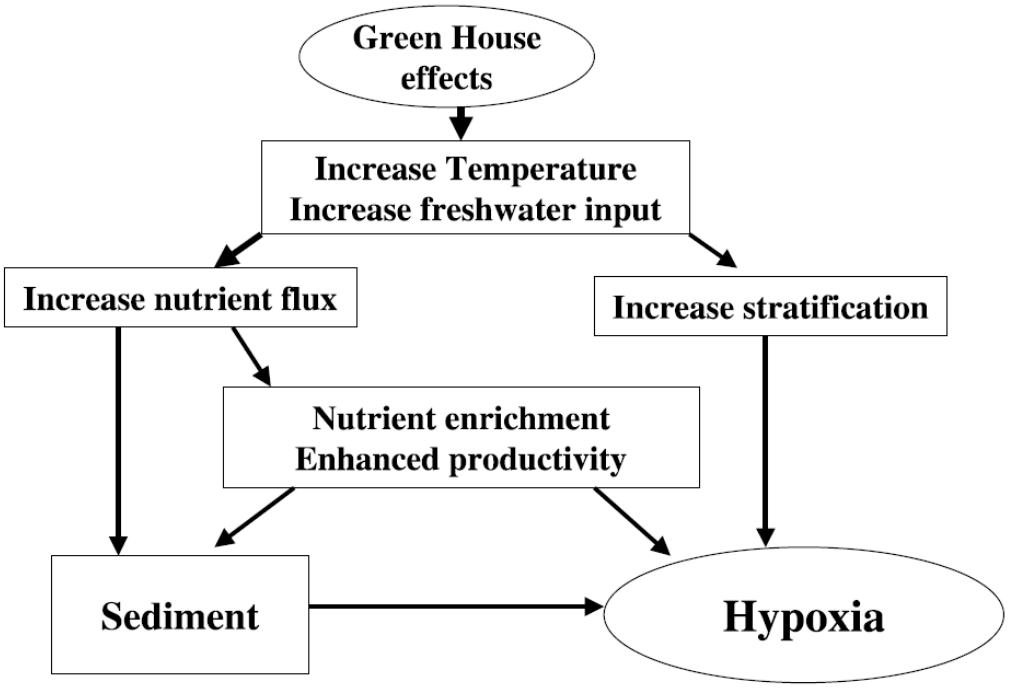


Figure 3. Possible effects of global warming on extension of hypoxia in coastal waters (Wu, 2002).

5. Eutrophication and oxygen depletion

Eutrophication is defined as overenrichment of waters by nutrients (nitrogen and phosphorus), which is a serious problem in many coastal ecosystems around the world (e.g. Diaz and Rosenberg, 1995; Tilman et al., 2001). This phenomenon is often directly

linked to the increase in human population growth and urbanization in the coastal drainage areas or to intensification of the agricultural activities in the river watersheds (Rabalais et al., 2010). Based on the human population growth and agricultural expansion, a 2.4 to 2.7 fold rise in the land-derived nutrients by 2050 has been projected (Tilman et al., 2001; Rabalais et al., 2010). This will result in mass phytoplankton blooms and causes oxygen depletion in the overlying water and sediment (Fig. 4). Although phytoplankton produces oxygen during the day via photosynthesis, oxygen depletion usually takes place at night when phytoplankton consumes more oxygen than produced by photosynthesis (Levin et al., 2009). During the phytoplankton bloom deposition (after red and green tides), the dead algal cells sink to the seabed where the first mineralization steps are performed by aerobic bacteria in the sediment (Wollast, 1998; Levin et al., 2009). As this process consumes oxygen, it reduces the dissolved oxygen concentration in the overlying water and in the sediment, especially under stratified conditions (Rabalais et al., 2010). The duration of oxygen stress in the overlying water is related to consumption of the oxygen by animals or biogeochemical processes and ventilation by water circulations (Conley et al., 2009).

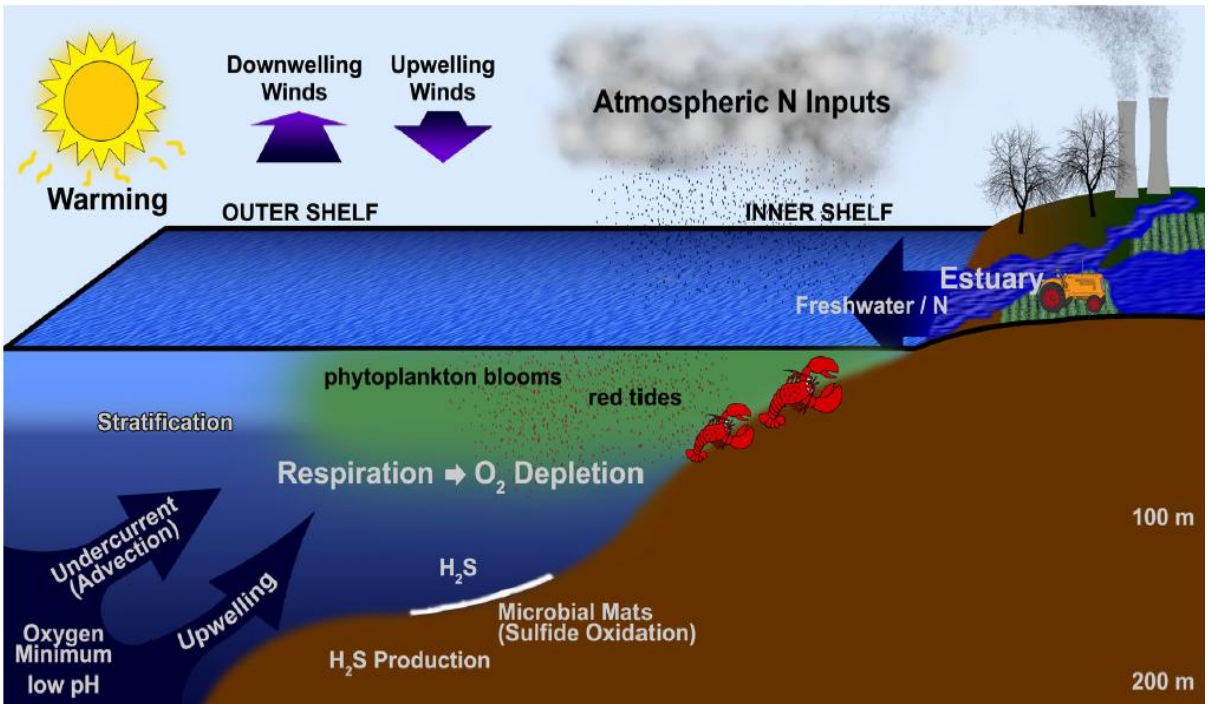


Figure 4. Schematic diagram illustrating eutrophication induced by riverine nutrient inputs (also other mechanisms) and phytoplankton blooms (Levin et al., 2009).

6. The effect of the oxygen depletion on fisheries and the coastal economy

Aquatic organisms need enough oxygen for their biological activities including feeding, growth and reproduction (Davis, 1975; Renaud, 1986; Wu, 2009). There is a variation in the responses of marine animals to low dissolved oxygen concentrations. However, dissolved oxygen concentrations below 2 mg l^{-1} (< 63 μmol l^{-1}) are sublethal or lethal for most of the organisms (Fig. 5) (Vaquer-Sunyer and Duarte, 2008; Levin et al., 2009).

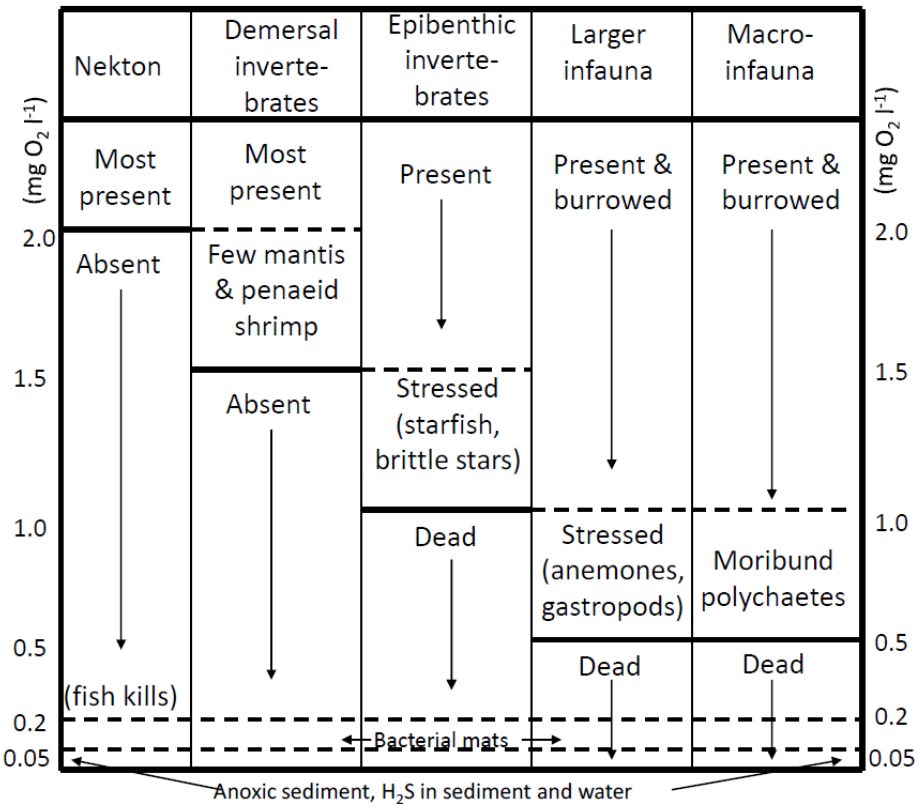


Figure 5. Responses of different marine animals to hypoxia (2 mg l^{-1} or < 63 µmol l^{-1}) in the Louisiana continental shelf (Levin et al., 2009).

It is known that oxygen depletion in the overlying bottom waters has an ultimate effect on food web dynamics through the negative impact on invertebrate behavior and predator-prey interactions, the survival of benthic animals and on the feeding activity of fishes as final consumers (Breitburg et al., 1997; Stachowitsch et al., 2007; Gobler et al., 2014). The negative effects of oxygen stress on reproduction, egg development, early life history and migration of economically important marine species have been well described (Baird et al., 2004; Ekau et al., 2009; Wu, 2009; Roegner et al., 2011). With regard to the importance of the coastal area for fisheries (Costanza, 1999), every disturbance in the marine coastal biodiversity including mass mortality, changes in

organism life cycles and reproduction of economically important marine species may lead to significant negative impacts on the economy (Breitburg et al., 1997; Baird et al., 2004; Turner et al., 2008; Zhang et al., 2010). Besides, biodiversity of the coastal areas is an important aspect to attract scuba divers and the development of marine ecotourism. It is reported that about 1.6 million tourists visit the Great Barrier Reef (GBR) region every year, and generate an income which is about three times more than commercial fishing income in this area (Harriott, 2002). In some countries like Thailand and Malaysia the dive tourism is a main income source for people living in the coastal area (Pascoe et al., 2014). Therefore, oxygen depletion and as a result mass mortality of marine animals can negatively influence ecotourism and the coastal economy.

7. Oxygen dynamics in coastal sediments and the relationship with benthic life

Marine coastal sediments are characterized by a rich and very productive benthic life and provide shelter and food for the benthic infaunal communities. Oxygen availability in the sediment is the result of the interplay between the influx of oxygen in the sediment (from the water column) and consumption (Cai and Sayles, 1996; Ziebis et al., 1996; Glud, 2008; Peña et al., 2010) and is generally limited to the upper centimeters (Rasmussen and Jørgensen, 1992) or even millimeters (Wenzhöfer and Glud, 2002; Braeckman et al., 2014). In the natural environment, the sediment oxygen content strongly depends on the oxygen concentration in the overlying water (Rasmussen and Jørgensen, 1992; Glud, 2008). Molecular diffusion is the main process for oxygen transport in the sediment (Glud, 2008; Middelburg and Levin, 2009) which is mediated by the combined effect of some other factors including sediment grain size, water

currents and macrofaunal activities (Berg et al., 2001; Vanaverbeke et al., 2011; Braeckman et al., 2014). For instance, in permeable sediments oxygenation is regulated by hydrodynamic forces. Advective pore-water flows can pump oxygenated water (240 µmol O_2 L^{-1}) till the upper 4 cm of sandy sediment (Huettel et al., 2014; Fig. 6). In non-permeable (finer) sediments macrofaunal and meiofauna activities (bioturbation and bio-irrigation) affect the sediment environment through transport of nutrients and oxygen from the surface-water interface to the deeper layers thereby altering the physicochemical properties of the sediment (Braeckman et al., 2010; Bonaglia et al., 2014; Huettel et al., 2014).

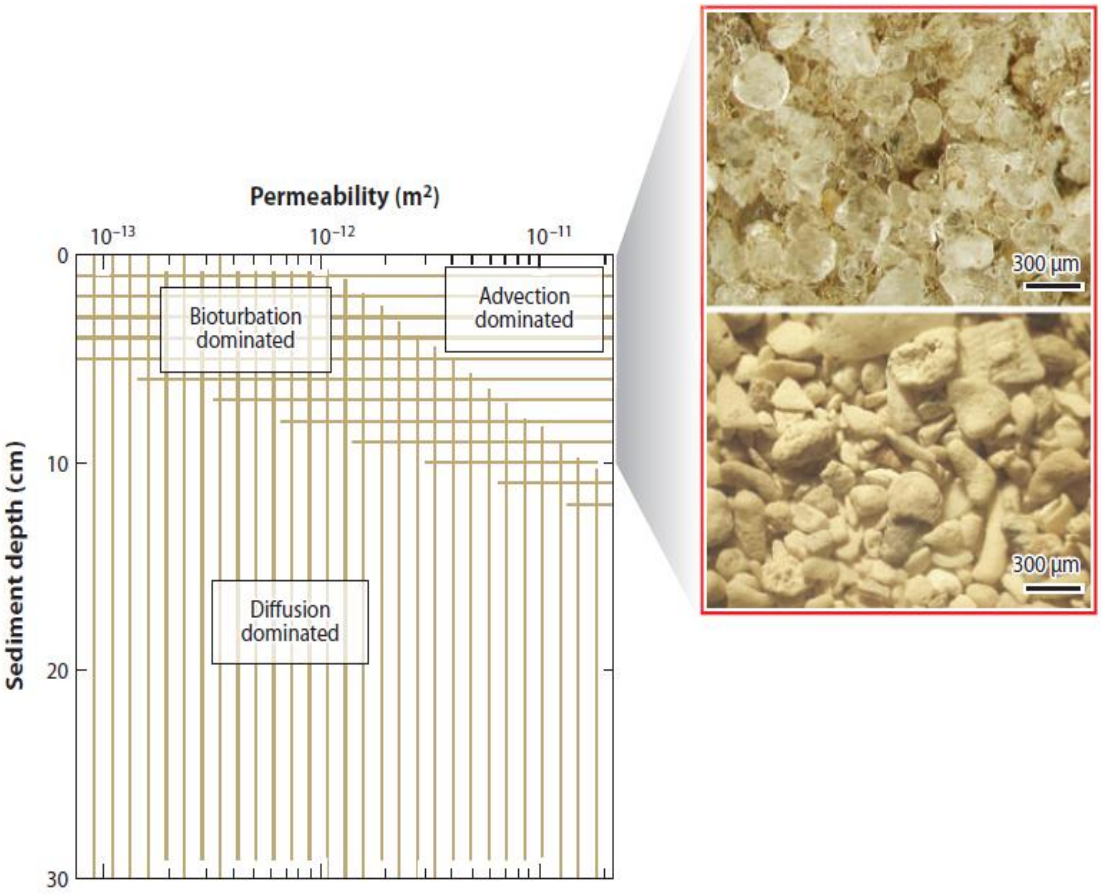


Figure 6. The ranges of dominant oxygen transport mechanisms in marine sediments (Huettel et al., 2014).

Direct oxygen consumption by benthic animal respiration and organic matter mineralization are two important reasons for oxygen depletion in the sediment. The oxygen consumption by macro and micro-organisms is different in different ecosystems. For example, 30 (in Autumn) till 40 (in Spring) percent of benthic community respiration was related to macrofaunal respiration in the strait of Øresund, Denmark (Kanneworff and Christensen, 1986). In the North Sea, bacteria were the most important oxygen consumers in the permeable sediments (Franco et al., 2010).

Following phytoplankton sedimentation, benthic mineralization processes (aerobic and anaerobic) play a key role in the recycling of nutrients and carbon in coastal sediments (Glud, 2008; Thomas et al., 2009). The top layer of the sediment is generally dominated by aerobic microorganisms. Oxygen serves as an electron acceptor in the degradation of organic matter in aerobic mineralization processes. Therefore, oxygen consumption by microbial activities leads to a decrease of the oxygen concentration with sediment depth (Katsev et al., 2007). Below the oxic zone further mineralization includes anaerobic processes such as nitrate/nitrite and sulphate reductions at different depths (Canfield et al., 1993; Katsev et al., 2007; Rabalais et al., 2010). In these layers, other electron acceptors than oxygen are utilized in mineralization processes (Kristensen and Holmer, 2001). The reduced chemical products (e.g. NH_4^+, Mn^{2+}, Fe^{2+} and H_2S) are then reoxidized in the oxic layers. Therefore, a part of the sediment oxygen consumption is resulting from the reoxidation of reduced chemicals (Fig. 7). In the marine sediments, about 20–30% of total organic carbon is oxidized aerobically in the fine sediment despite the shallow penetration of oxygen (Canfield et al., 1993; Katsev et al., 2007).

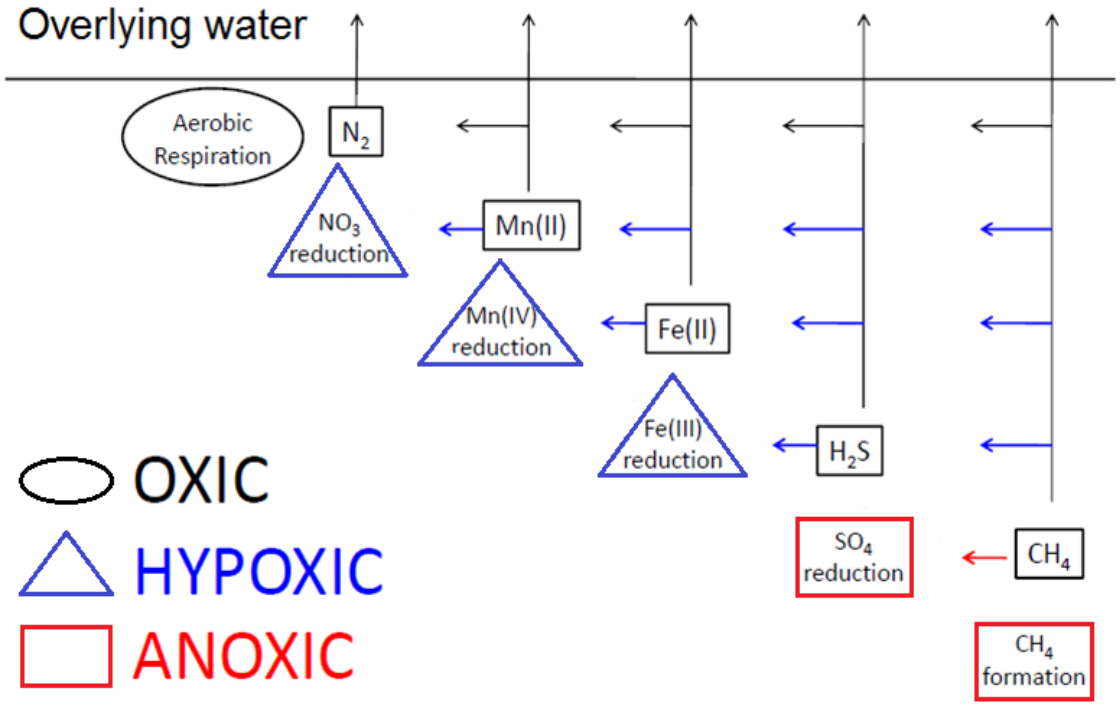


Figure 7. Organic matter mineralization in oxic (O_2 concentration > 63 µmol l^{-1}), hypoxic (63 µmol l^{-1} > O_2 concentration > 0 µmol l^{-1}) and anoxic (O_2 concentration = 0 µmol l^{-1}) marine sediments (Middelburg and Levin, 2009).

8. The effect of oxygen depletion on marine benthic biodiversity (including aspects related to recovery processes)

Hypoxia and anoxia generally occur in the bottom water and sediment (Diaz and Rosenberg, 1995) and as a consequence benthic communities, from the individual organism to whole ecosystems, can be extremely subjected to oxygen stress, especially in the coastal areas (Riedel et al., 2014). With regard to the shallow oxygen penetration depth in coastal sediments (Wenzhöfer and Glud, 2002; Braeckman et al., 2014), deeper sediment layers are anoxic and consequently, sulphidic sediments have a worldwide distribution (Diaz and Rosenberg, 1995; Wenzhöfer and Glud, 2002). Responses of benthic communities (macro- and meiofauna) to oxygen stress depend

on the period and intensity of the oxygen depletion (Modig and Olafsson, 1998; Levin et al., 2009; Vaquer-Sunyer and Duarte, 2010). In general, larger taxa (macrofauna) are more sensitive than small taxa (meiofauna) to oxygen stress (Levin et al., 2009; Van Colen et al., 2009).

Such stress can lead to behavioral reactions (change in location, direction and body posture) as observed in some macrofaunal communities (Diaz and Rosenberg, 1995; Riedel et al., 2014). An upward migration from low oxygenated sediment layers to the water column was reported for some mobile macrofauna species as an initial response to decreasing oxygen concentration (Diaz and Rosenberg, 1995). Hypoxia can not only cause changes in behavioural reactions of macrofaunal organisms (Riedel et al., 2014), it can decrease their density and species richness (Riedel et al., 2012, 2014), total biomass of the macrofaunal communities (Sturdivant et al., 2013) and finally result in mass mortality (Stachowitsch, 1984; Stachowitsch et al., 2007).

The response of meiofauna taxa to oxygen stress is species-specific and related to their evolutionary history and lifestyles (Wetzel et al., 2001). Generally, copepods are the most sensitive and Foraminifera are the most resistant meiofaunal group to oxygen stress in coastal sediments (De Troch et al., 2013; Grego et al., 2014; Langlet et al., 2014). For example, the results of an experimental study in the Adriatic Sea indicated a sharp decrease in density (90%) and species richness (80%) of a copepod community after 23 days exposure to anoxia (Grego et al., 2014) while it was only 33 and 11 % for a Foraminifera community (Langlet et al., 2013; Fig. 8).

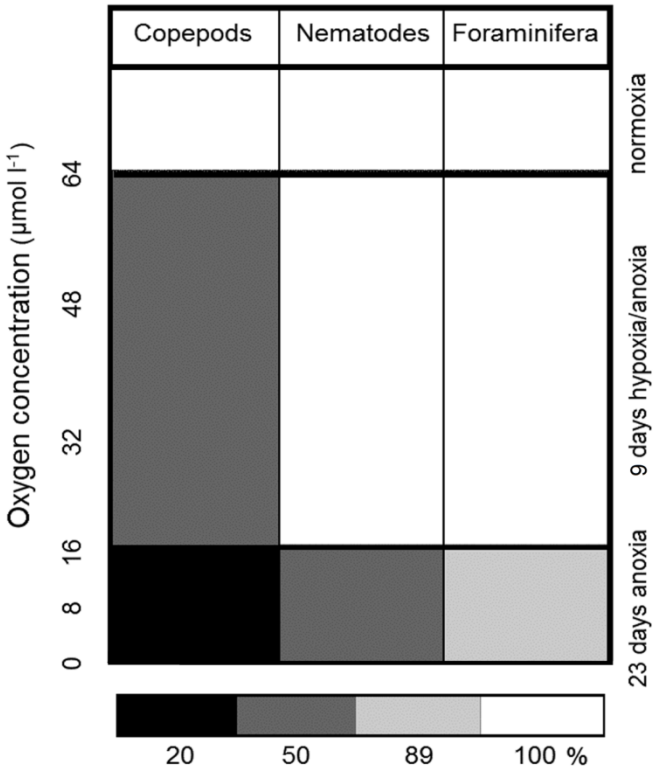


Figure 8. Responses of meiofauna (species richness) to severity and duration of oxygen stress in the Gulf of Trieste, the northern Adriatic Sea (Langlet et al., 2013; Grego et al., 2014 and the present thesis).

Drastic changes in meiofaunal community structure and vertical distribution patterns were also observed when oxygen stress lasted longer than 2 months (Wieser and Kanwisher, 1961; Moodley et al., 1997; Wetzel et al., 2002). An upward migration from low oxygenated sediment layers to the water column was reported for meiofauna as well (Wetzel et al., 2001; Duijnstee et al., 2003). Several adaptation mechanisms such as decreased feeding rates (Steyaert et al., 2007; De Troch et al., 2013) and reproduction activity (Jensen, 1995), a reduction in respiration rate (Braeckman et al., 2013) and the ability to switch to anaerobic metabolism (Moodley and Hess, 1992) help some species to survive weeks or months under hypoxic and anoxic conditions.

The response of a meiofauna community to oxygen stress is also related to the exposure history (frequency of oxygen stress) (Modig and Olafsson, 1998; Wetzel et al., 2001; Baustian and Rabalais, 2009; Levin et al., 2009). Benthic fauna which are exposed to episodic oxygen stress (more than one time per year) are better adapted to survive. Their populations can increase when the density of other common species decreases (Rabalais et al., 2001; Baustian and Rabalais, 2009). It is noticeable especially for those groups which have seasonal life cycles (not enough time for compelet recruitment; Diaz and Rosenburge, 2008). The post-hypoxia communities are generally dominated by one or a few oxygen tolerant species (Levin, 2003) occurring in high densities (Baustian and Rabalais, 2009) and low species richness (Diaz and Rosenburge, 2008).

Recovery of the benthic fauna after oxygen stress is a complex and long process (Diaz and Rosenberg, 1995; Wetzel et al., 2001, 2002; Levin et al., 2009). In short, negative effects (mass mortality of organisms) of oxygen depletion on marine benthic communities are much faster than an eventual recovery phase ("rapid death, slow recovery": Stachowitsch, 1991, 2007). Faunal recolonization and recovery are related to the recovery of the sediment biogeochemistry (Middelburg and Levin, 2009) and usually start when the dissolved oxygen concentration in the water column reaches at least 2.1 ml l^{-1} or 3 mg l^{-1} (Steckbauer et al., 2011). Recovery of sediment biogeochemistry after oxygen depletion is still poorly understood (Middelburg and Levin, 2009). After oxygen stress, the sediment is full of reduced substances, and therefore the new oxygen re-entering the sediment is consumed by reoxygenation of these reduced substances (Rabalais et al., 2014) which significantly prolongs the period of unfavorable sediment

conditions for the benthic fauna (Bastviken et al., 2004; Middelburg and Levin, 2009; Soetaert and Middelburg, 2009; Peña et al., 2010).

There is also a strong relationship between biogeochemical recovery, macrofaunal recovery, and meiofaunal community development after oxygen stress (Van Colen et al., 2009). Bioturbating and bio-irrigating activities of macrofauna increase benthic mineralization (Meysman et al., 2006; Middelburg and Levin, 2009); transfer oxygen to deeper sediment layers (Braeckman et al., 2010) and probably enhances recovery of meiofauna (Van Colen et al., 2009). However, recolonization and recovery of the benthic community mainly occur through migration (with currents or by active movements) from surrounding, non-impacted environments rather than by reproduction of surviving individuals (Guerrini et al., 1998; Wetzel et al., 2001). Nematodes have been reported as the most successful meiofauna group in recovery phase after anoxic stress (Travizi, 1998). Nematode genera based on their life history strategies can be defined a c–p (coloniser–persister) score ranging between 1 (colonisers: short generation time, high reproduction rate, high colonisation ability and tolerant towards pollution and disturbance) and 5 (persisters: long life cycle, low reproduction potential, sensitive to pollution and disturbance). Genera with a c–p score of 2, 3 or 4 are intermediate between colonisers and persisters (Bongers, 1990, 1991).

9. Marine free-living nematodes and oxygen stress

The focus of this PhD research is on the response of benthic nematodes from coastal areas to varying levels of oxygen stress. Marine free-living nematodes are the most abundant metazoans in marine sediments. Their numbers can reach millions of individuals per square meter of sediment (Heip et al., 1985; Giere, 2009), penetrating

down to 105 cm below the sediment-water interface in sandy beaches (Heip et al., 1982). The highly abundant nematode populations comprise different feeding guilds from bacteria feeders to predators (Wieser, 1953; Moens and Vincx, 1997), they can considerably influence ecological processes such as nutrient cycling, organic matter mineralization, bioturbation of sediments (Nascimento et al., 2012; Moens et al., 2013; Bonaglia et al., 2014) and are important in the energy transfer toward higher levels in the benthic food webs (Giere, 2009; Balsamo et al., 2010; Moens et al., 2013). Due to their sensitivity to changes in the environment, the high number of species and individuals, their short lifecycles and thus rapid generation turnover, and the lack of pelagic life stages, they are suitable bio-indicators of anthropogenic disturbance in marine ecosystems (Balsamo et al., 2010; Semprucci et al., 2015).

The horizontal and vertical distribution of free-living nematodes is controlled by interactions of several abiotic and biotic factors. For example, sediment grain size can control the distribution of nematode communities as it is related to sediment organic matter content (food source) and oxygen availability (Vanaverbeke et al., 2011). It has been reported that macrofaunal organisms shape nematode communities through their bioturbation and bio-irrigation activities by transferring food and oxygen from the sediment surface to deeper layers (Braeckman et al., 2011b). However, interactions between the effect of abiotic and biotic factors and responses of nematodes are complex (Giere, 2009; Van Colen et al., 2015).

As most of marine nematodes have aerobic respiration (Wetzel et al., 2001; Steyaert et al., 2005), oxygen plays an essential role in their spatial occurrence within the sediment depth: most of the nematodes do occur in the top layers of the sediments where both

oxygen and food are available (Giere, 2009; Vanaverbeke et al., 2011). However, some marine nematode species like *Terschellingia communis*, *Metachromadora vivipara* and *Sabatieria pulchra* live in anoxic/sulphidic areas (Ott and Schiemer, 1973; Polz et al., 1992; Hendelberg and Jensen, 1993; Hentschel et al., 1999; Vanaverbeke et al., 2004; Steyaert et al., 2007; Braeckman et al., 2011b; Vanaverbeke et al., 2011). Resistance of nematodes to hypoxic/anoxic conditions is species-specific and related to the duration, exposure history and the natural adaptations of the species to live in oxygen-stressed environments (Modig and Olafsson, 1998; Hentschel et al., 1999; Travizi, 2000; Wetzel et al., 2001; Steyaert et al., 2007).

Different adaptation mechanisms help marine nematodes to cope with temporary hypoxic/anoxic conditions. Generally long and slender nematodes are more tolerant to anoxia (Soetaert et al., 2002). Some earlier studies indicated that nematode total density, diversity, community composition and vertical distribution is not negatively affected by short-term (≤ 1 week) anoxia (Guerrini et al., 1998; Steyaert et al., 2005). Decreases in feeding activity and respiration rate (metabolism) have been reported as adaptation mechanisms to overcome oxygen stress in marine nematodes (Ott and Schiemer, 1973; Steyaert et al., 2007; Braeckman et al., 2013). Longer periods of hypoxia/anoxia cause a decrease in nematode density and changes in the community structure (Wetzel et al., 2001; Steyaert et al., 2007; Gambi et al., 2009; Van Colen et al., 2009) as well as a prolongation of the generation time (Jensen, 1995).

Anoxia in marine sediments is often accompanied by the presence of hydrogen sulfide (H_2S) which is toxic for most animals. The toxicity of H_2S is mainly due to the reversible inhibition of the cytochrome c oxidase, a key enzyme in aerobic respiration (Vismann,

1991). Vaquer-Sunyer and Duarte, (2010) indicated a reduction in survival times under hypoxia by an average of 30% in marine benthic communities when H_2S was present. Responses of benthic nematode communities to hypoxia and the presence of H_2S are species-specific (Modig and Olafsson, 1998; Gambi et al., 2009; Levin et al., 2009). Even among cryptic species of *Halomonhystera disjuncta*, different levels of tolerance to H_2S were reported (Van Campenhout et al., 2014). Nevertheless, some nematode species can survive in this condition. For example, density of *Metachromadora vivipara* even increased in anoxic/sulphidic conditions (Steyaert et al., 2007). Different adaptation mechanisms help them to survive in this toxic environment. Some marine nematodes also have symbiosis with sulfur-oxidizing bacteria which decoratively ornament their cuticles, and enable them to live in deeper sediment layers under the chemocline (Ott et al., 2004). Endosymbiotic sulfur-oxidizing bacteria have also been reported for some marine nematode genera including *Astomonema* (Giere et al., 1995; Musat et al., 2007) and *Parastomonema* (Kito, 1989). Conversion of H_2S to elemental sulphur which temporarily reduces the concentration and toxic effect of H_2S was also reported in *Oncholaimus campylocercoides,* a marine nematode (Thiermann et al., 2000). Also, iron has been found in some tissues of nematodes living in anoxic/sulphidic conditions, and it has been suggested that it binds reduced sulphur (Giere, 2009).

The Oxygen Minimum Zone (OMZs) is defined as regions where oxygen concentrations are less than 22 µM (< 0.5 ml l^{-1}). These zones generally occur at depths of about 200 to 1,000 meters, depending on local circumstances (Levin et al., 2003). Where OMZs intercept the continental margin or seamounts, they have large effects on benthic assemblages (Levin et al., 2003). Apart from oxygen concentration, metazoan

communities in these areas are also affected by gradients in organic matter content (Levin and Gage, 1998; Neira et al., 2001; Singh and Ingole, 2016). Among the metazoan meiofauna living in OMZs, nematodes are known to be tolerant group of low oxygen concentrations and high organic matter content (Levin et al., 1991; Cook et al., 2000; Neira et al., 2001). Some studies indicated that nematodes could resistancy in low oxygen concentration even till 1.44 µmol l^{-1} in OMZs (e.g. Neira et al., 2001; Neira and Decraemer, 2009; Urkmez et al., 2015). It seems that food availability has a greater impact on nematode communities than oxygen concentration in OMZs (Cook et al., 2000; Neira et al., 2001). This can be supported by Muschiol et al. (2015) who indicated that bacterivorous aquatic nematodes could survive in long term hypoxia (one year) as long as the microbial food source was available.

10. Objectives

The overall aim of this PhD research was to increase the knowledge on the effect of oxygen depletion (severity and duration) in the water column on the marine free-living nematodes living in coastal sediments. We expected stronger negative effect with increasing severity (from hypoxia to anoxia) and duration (from 1 day to 307 days) of oxygen stress. This thesis focused on the coastal area (intertidal and subtidal) being more influenced by oxygen depletion than the open oceans due to a wide range of human activities related to eutrophication and ocean warming (Gilbert et al., 2010).

Specific objectives of the PhD thesis:

- To evaluate and compare the effect of short-term hypoxia (1 and 7 days) on structural characteristics (density, diversity, vertical distribution and community

structure) of different nematode communities (sampled from three different sediment types).

- To evaluate the response of nematode communities to short and long-term anoxia, and the subsequent recovery of the nematode communities.

- To evaluate the effect of short-term hypoxia (6 days) on the feeding activities of the intertidal nematodes.

- To integrate the results with the existing knowledge to look for general patterns on the response of nematode communities to oxygen stress.

Both *in situ* and laboratory experiments were conducted in three different areas and sediment types, in order to evaluate the sediment-related responses of the nematodes to oxygen stress (Fig. 9). In this thesis, for the first time, both short- and long-term anoxia were tested and evaluated together with the recovery of the nematode communities after long periods of oxygen stress. These investigations are crucial to understand the responses in nature and to better evaluate the data gained through many monitoring programs of coastal ecosystems, using nematodes as bio-indicator for oxygen stress.

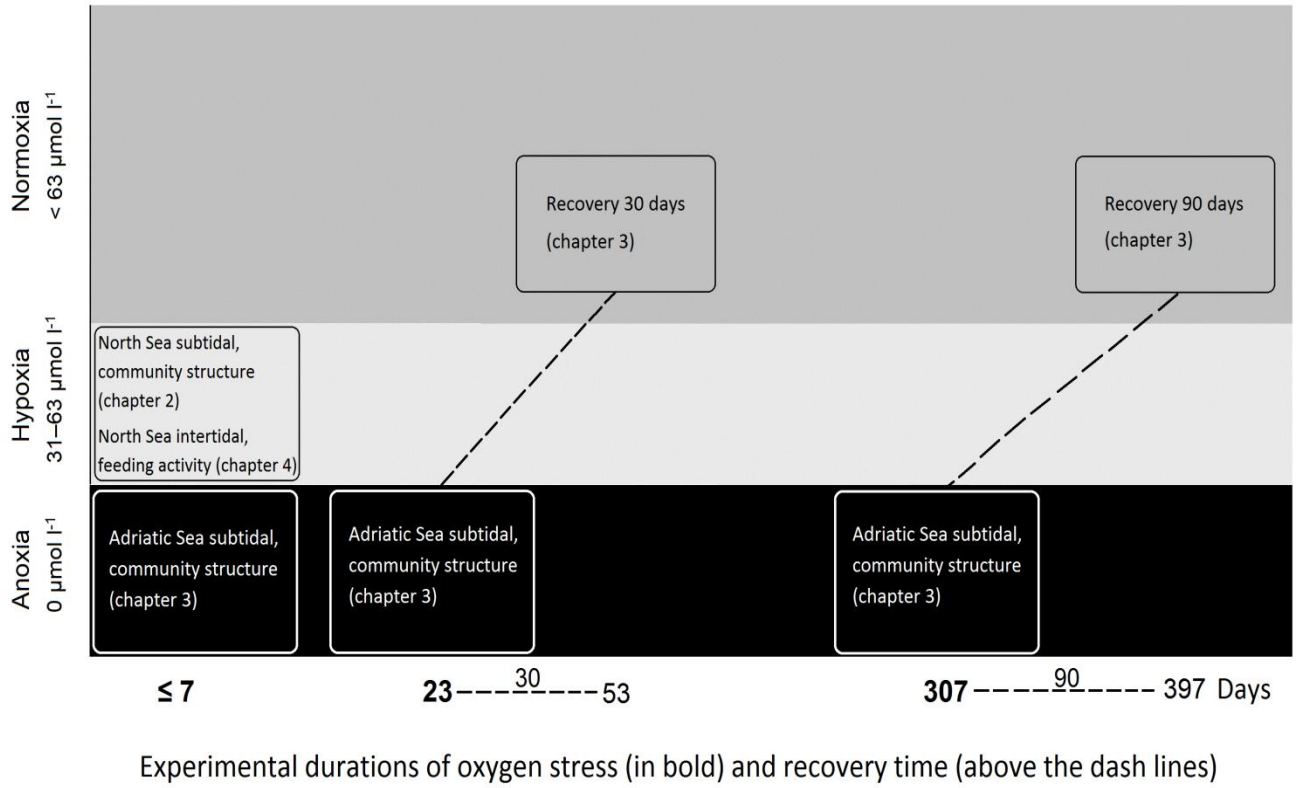


Figure 9. Schematic overview of the potential effect of oxygen stress (severity and duration) on nematode community characteristics and feeding activity which were studied in the present PhD thesis.

11. Study areas

In this PhD thesis, the responses of nematode communities to hypoxic/anoxic conditions were experimentally investigated in soft-bottom sediments in three different European countries (Fig. 10). The overall biological specifications and oceanographic aspects of the study areas are described in the following paragraphs.

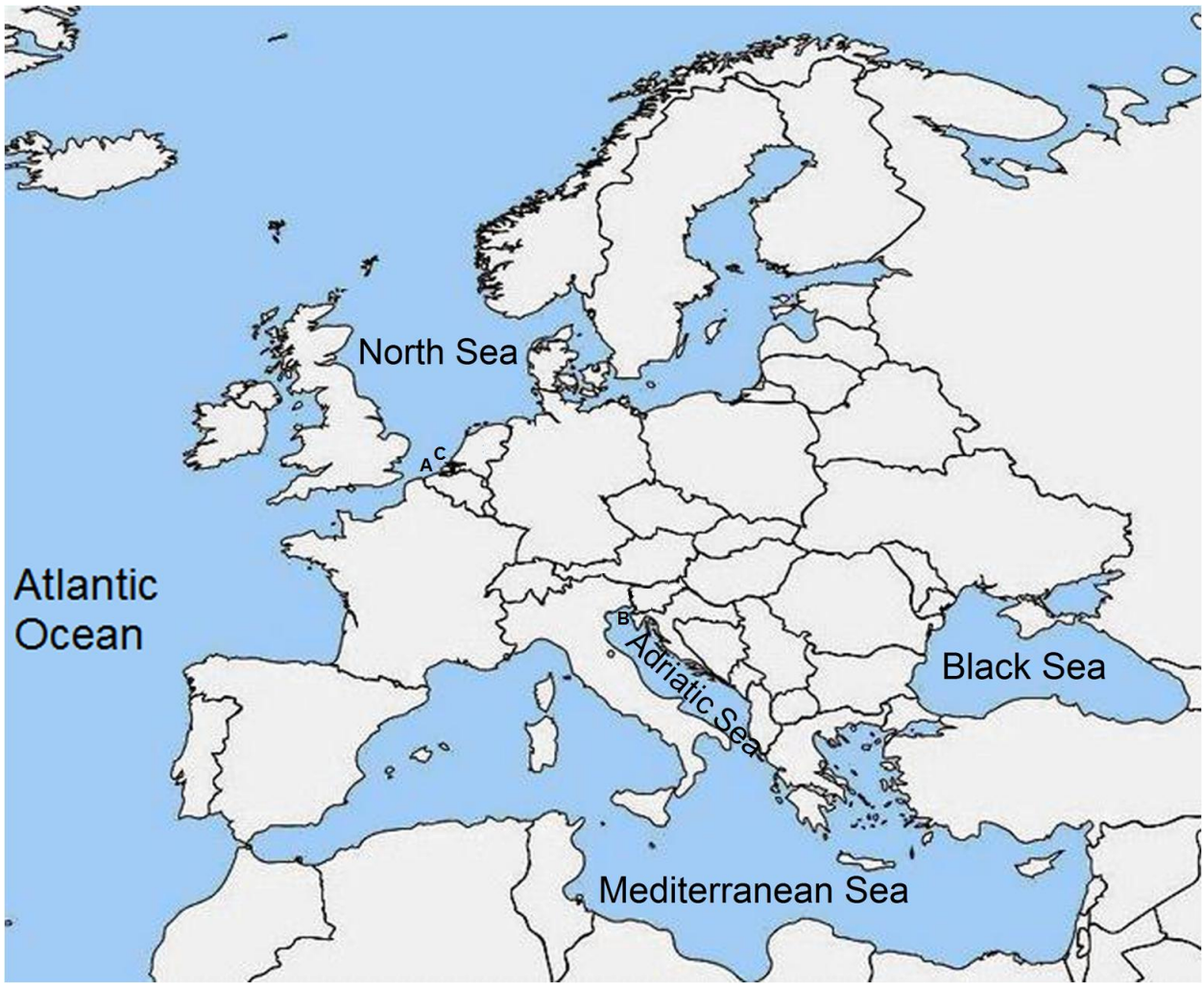


Figure 10. Overview map with all study areas. A: Belgian part of the North Sea (Belgium), B: The northern Adriatic Sea (Slovenia) and C: Westerschelde Estuary (SW the Netherlands).

11.1 Belgian part of the North Sea (Belgium)

The Belgian Part of the North Sea (BPNS) is a rather small and shallow shelf area (3600 km², average depth about 20 m), characterized by a highly variable and complex topography (i.e. presence of sandbanks) and by different sediment types with the median grain size ranging between 88 and 494 µm (Degraer et al., 2006; Verfaillie et al., 2006; Vanaverbeke et al., 2011). This variation in habitats is responsible for a high benthic (macro- and meio-) biodiversity and supports the presence of different

communities (Van Hoey et al., 2004; Degraer et al., 2006; Vanaverbeke et al., 2011). This area is heavily affected by different human activities including fishing, sand and gravel extraction, change in coastal landscape, ocean warming and eutrophication (Degraer et al., 2006).

Peak phytoplankton blooms on the BPNS occur in mid-May (dominated by *Phaeocystis*) and in July and August (dominated by diatoms) (Rousseau et al., 2002) followed by mass sedimentation of phytoplankton in May and June (Lancelot et al., 2005; Provoost et al., 2013; Braeckman et al., 2014). The increasing sediment organic matter content can influence the structural and functional diversity of nematode communities (Vanaverbeke et al., 2004; Franco et al., 2008a) as a result of changes in the biogeochemical characteristics of the sediment. Mineralization rates of the organic matter are different in different sediment types (Braeckman et al., 2014). In fine-grained sediments mineralization is related to temperature and is more efficient in late summer when temperature is highest (Provoost et al., 2013). In permeable sediments, the high oxygen availability due to advective pore water flows could increase mineralization rate even more than muddy and fine sandy sediments (Gao et al., 2012). Sediment grain size is known as an important factor affecting nematode community characteristics (Vanaverbeke et al., 2011). The diversity in sediments allowed us to test the response of very different nematode communities that are subjected to the same global environmental conditions (salinity, water temperature, timing and magnitude of phytoplankton bloom) to a short-term hypoxia event.

11.2 The northern Adriatic Sea (Slovenia)

The Northern Adriatic Sea (Gulf of Trieste) is a recognized area for seasonal oxygen depletion events (Malej and Malacic, 1995; Diaz and Rosenberg, 2008; Giani et al., 2012). The Gulf of Trieste covers an area of about 600 km^2 and is a semi-enclosed shallow marine basin with high riverine inputs of nutrients and organic matter in the northern part of the Adriatic Sea (Giani et al., 2012). Long-term eutrophication results in massive phytoplankton blooms and sedimentation of these blooms, together with water column stratification, results in late summer hypoxia and anoxia (Justic, 1987; Malej and Malacic, 1995; Giani et al., 2012). However, the frequency of these low-oxygen events decreased after the 1990s as a result of reduced continental nutrient inputs (Giani et al., 2012). The benthic macrofauna community in this Gulf is dominated by brittle stars, sponges and tunicates (Fedra et al., 1976; Ogorelec et al., 1991) while nematodes are the prevailing meiofaunal group (Travizi, 1998, 2000). As a consequence of oxygen depletion, mass mortality in all animal groups was reported before (e.g. Stachowitsch, 1984; Hrs-Brenko et al., 1994), although the meiofaunal was rather tolerant and resilient (Travizi, 1998). Due to repeated bottom water oxygen depletion as a result of human activities, this Gulf is an ideal setting for *in situ* investigations on the effect of hypoxia/anoxia and recovery on different benthic communities which are already adapted to these circumstances (Riedel et al., 2012, 2014; De Troch et al., 2013, Grego et al., 2014; Langlet et al., 2014).

11.3 Paulina intertidal flat, Westerschelde Estuary (SW the Netherlands)

The Westerschelde estuary is the lower part of the Schelde River, and is located in the south western part of the Netherlands (province of Zeeland). It is an important shipping path to the Port of Antwerp, Belgium. This estuary is a highly dynamic environment due to hydrodynamic forces (mainly the tides), characterised by animal communities with a low biodiversity and consisting of species adapted to daily changes in salinity and temperature (Heip, 1988). Long-term trends (1965–2002) in dissolved inorganic nutrients in the tidal part of the Scheldt estuary showed fluctuating patterns. Annually averaged concentrations of dissolved inorganic nutrients (Si, N and P) significantly declined after mid-1970s. In the early 1970s, high loadings of ammonium and organic matter caused oxygen depletion (Heip, 1988; Baeyens et al., 1998; Soetaert et al., 2006) but with a decrease of the ammonium input in the 1990s, an increase in oxygenation was visible from 1995 onwards (Soetaert et al., 2006).

The Paulina intertidal mudflat is located in the polyhaline (average salinity between 24 and 32) part of the Westerschelde estuary (Moens et al., 2002). The mudflat has a gentle slope and a mean tidal range of 3.9 m, with a semidiurnal regime. In this area, different sediment types, hydrodynamics, tidal currents and shore vegetation provide several habitats (Van Colen et al., 2010a). Nematodes are dominant in the meiofauna community in the intertidal area and their highest density is reported in the upper centimeter layer (Soetaert et al., 1994; Steyaert et al., 2003; Van Colen et al., 2009). Therefore, we investigated the effect of short-term hypoxia on the nematode community of the upper centimeter layer, which have been suggested to be more sensitive to oxygen stress (Wetzel et al., 2001; Giere, 2009).

12. Outline of the thesis

Apart from Chapter 1 (general introduction) and Chapter 5 (general discussion), the chapters of this thesis represent complete research articles which were either published (Chapter 2 or 3) or submitted (Chapter 4). The references have been collected in a single list after the appendices. Addenda I and II consist of supplementary materials (hereafter referred to as S) to Chapters 2 and 3.

It has been suggested that the ability of nematode communities to survive oxygen stress depends on the nature of the community: communities that are regularly subjected to oxygen stress are better adapted than others (Vanaverbeke et al., 2004, Vanaverbeke et al., 2011). In **Chapter 2,** we subjected nematodes from continuously oxygenated sediments and sediments partly subjected to oxygen stress to similar levels of hypoxia. An experiment was carried out in the lab, using nematode communities sampled at three different stations in the BPNS. As they originate from the same area, the global temporal environmental settings (temperature, timing and magnitude of the bloom) are identical. We test the hypothesis (H0) that duration of hypoxia does not affect the nematode community characteristics in the different sediment types. This chapter was published as Taheri, M., Braeckman, U., Vincx, M., Vanaverbeke, J., 2014. Effect of short-term hypoxia on marine nematode community structure and vertical distribution pattern in three different sediment types of the North Sea. Marine Environmental Research. 99,149-159.

Responses of marine benthic communities to oxygen stress are species-specific and related to the duration of oxygen stress (Modig and Olafsson, 1998, Levin et al., 2009). In **Chapter 3**, we investigated how the duration of oxygen stress affected (1) the

response of nematode communities, and (2) the recovery patterns of nematode communities after re-oxygenation of the overlying water. Therefore, an *in situ* experiment was carried out in the Gulf of Trieste, Northern Adriatic Sea. Anoxia was created artificially by three underwater benthic Plexiglas chambers at a depth of 24 m. Treatments lasted for 2, 23 and 307 days. Control samples (Normoxia) were taken outside the chambers (4-5 m further). After opening the chambers, recovery cores were taken after 7 days (Anoxia 2D), 30 days (Anoxia 23D) and 90 days (Anoxia 307D). We tested the hypotheses (H0) that, i) the duration of in situ anoxia does not affect the nematode community characteristics (density, diversity, vertical distribution and feeding type contributions), and ii) the recovery of the nematode community is not related to the duration of anoxia. The results of this experiment were published as Taheri, M., Grego, M., Riedel, B., Vincx, M., Vanaverbeke, J., 2015. Patterns in nematode community during and after experimentally induced anoxia in the northern Adriatic Sea. Marine Environmental Research. 110, 110-123.

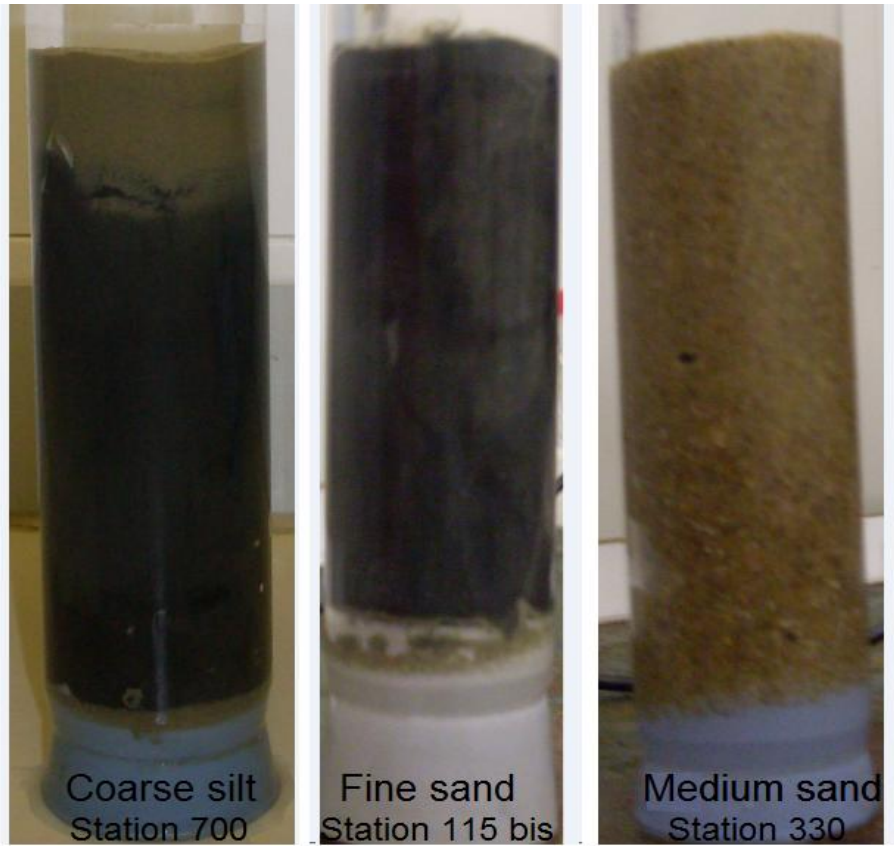
Our results from the previous Chapters (2 and 3) indicated a tolerance of the nematode community to short-term hypoxia/anoxia. Decrease in respiration rate (metabolism) could be an adaptation to cope with oxygen stress (Ott and Schiemer, 1973, Braeckman et al., 2013), while reduced food consumption in anoxic environments was reported as well (Steyaert et al., 2007). A reduction in food uptake can result in a decreased respiration. Therefore, in **Chapter 4** the effect of short-term hypoxia (6 days) on the feeding activity of dominant nematode genera was investigated in a laboratory experiment using ^{13}C pre-labelled diatoms as an additional food source. Nematodes were sampled from the Paulina intertidal flat in the Westerschelde estuary, south-west

Netherlands and four dominant genera were selected as representatives of different feeding types. In this experiment, we tested the hypothesis that (H0) short-term hypoxia (6 days) does not affect the feeding activity of nematode communities. This result can evaluate the real impact of oxygen stress on a part of the food web of estuarine benthic ecosystems (the free-living marine nematodes). The results of this chapter is in preparation as Taheri, M., Giunio, M., De Troch, M., Vincx, M., Vanaverbeke, J., Effect of short-term hypoxia on the feeding activity of abundant nematode genera from an intertidal mudflat.

In the last chapter (**Chapter 5**), the main conclusions are summarized, discussed and compared with other studies and recommendations for future research (e.g. effect of hydrogen sulphide on nematode community, effect of oxygen stress on feeding activity and reproduction and possibility of nitrate respiration) are provided.

Chapter 2

Effect of short-term hypoxia on marine nematode community structure and vertical distribution pattern in three different sediment types of the North Sea



Modified from the following publication:

Mehrshad Taheri, Ulrike Braeckman, Magda Vincx and Jan Vanaverbeke. 2014. Effect of short-term hypoxia on marine nematode community structure and vertical distribution pattern in three different sediment types of the North Sea. Marine Environmental Research, 99: 149-159.

ABSTRACT

In the natural environment, drifting algal mats can induce hypoxia (oxygen < 64 µmol l^{-1}) in the sediment within a short time (3-9 days) and as a result decrease density and change in the diversity of benthic animals (Norkko and Bonsdorff, 1996; Arroyo at al., 2012). In the present study the responses of nematode communities to short-term hypoxia (1 and 7 days) were investigated in three North Sea stations with different sediment types (coarse silt, fine sand and medium sand). In the field, nematode density, diversity, vertical distribution and community structure differ among the stations. In the laboratory, oxic and hypoxic treatments were established for 1 and 7 days for all sediment types. Comparison between field control and oxic day 1 treatments showed that experimental sediment handling did not affect nematode characteristics. Our results revealed that short-term hypoxia did not affect total density, diversity, community composition, vertical density profiles (except in the fine sand) and densities of five dominant species in all sediment types.

Keywords Hypoxia, Marine nematodes, Community structure, Vertical distribution, Sediments, North Sea.

1. Introduction

Coastal hypoxia (oxygen concentration < 63 µmol l^{-1} or < 2 mg l^{-1}) is increasing worldwide due to natural and anthropogenic impacts (Diaz and Rosenberg, 2008). The number of coastal areas where hypoxia has been reported increased exponentially with 5.5% $year^{-1}$ from 1916 till 2006 (Vaquer-Sunyer and Duarte, 2008).

While coastal hypoxia is an environmental phenomenon occurring mainly in the water column, low oxygen concentration in the bottom water affects the benthic ecosystem as well, as increasing organic matter accumulation rates, decreasing macrofaunal bioirrigation and bioturbation activities and changes in sediment biogeochemistry in hypoxic environments have been reported (Smith et al., 2000; Diaz and Rosenberg, 2008; Middelburg and Levin, 2009). While oxygen penetration depth in sediment is generally limited to the upper centimetres (Rasmussen and Jørgensen, 1992) or even millimetres (Wenzhöfer and Glud, 2002), marine hypoxia can affect the community structure and the vertical distribution of meiofauna (Gambi et al., 2009; Levin et al., 2009). The response of free-living marine nematodes to hypoxia is not uniform (Modig and Olafsson, 1998; Wetzel et al., 2001). Research on intertidal nematode communities showed that they prefer oxic conditions (Steyaert et al., 2005). Hypoxic conditions resulted in mortality of about one third of the intertidal nematode communities and reduced the activity level (measured as feeding activity) of the surviving nematodes after 2 weeks (Steyaert et al., 2007). In the northern Baltic Sea, short-term (6 days) hypoxia caused a decline in nematode abundance coinciding with a change in community structure and functional diversity (Arroyo et al., 2012). Mortality of about 75% of the nematode community was observed within 9 days of laboratory-induced

hypoxia as well (Wetzel et al., 2001). Furthermore, response of nematodes from different sediment depths to overlaying water oxygen stress is different. Within five days, 90 % mortality was observed in non-thiobiotic nematodes which are living in top centimetres of sediment, while thiobiotic nematodes (i.e., *Sabatieria pulchra*) from deeper sediment layers survived up to seven weeks in an anoxic treatment (Jensen, 1984). In a tidal flat of the Westerschelde estuary (the Netherlands), long-term hypoxia (40 days) drastically changed nematode community structure and reduced diversity and abundance of some dominant species (Van Colen et al., 2009). In the Mediterranean Sea, nematode species richness was not affected by long-term hypoxic-anoxic conditions (5 months), though species composition and trophic structure changed significantly (Gambi et al., 2009). Nematode survival against hypoxia was observed in the Black Sea (Muresan and Gomoiu, 2012) as well and both studies revealed that nematodes belonging to the genus *Sabatieria* were the most frequent and abundant group in hypoxic conditions. Moodley et al. (2000) showed that the vertical distribution of nematodes was primarily related to macrofaunal activity, rather than to sediment oxygen concentration. In the Gulf of Oman, nematode abundances were more affected by food quality than by sediment oxygen concentration too (Cook et al., 2000).

The contrasting results mentioned above might be caused by the lack of spatial resolution in most of the mentioned studies. Apart from oxygen concentration in the bottom water, nematode community structure is known to be affected by sediment conditions as well (Vanaverbeke et al., 2011). While most studies on the effect of hypoxia on nematodes did not take into account spatial variability, in the present study we hypothesis that the effect of hypoxia depends on the characteristics of the ambient

nematode community, which is in turn affected by sediment granulometry. Under low oxygen conditions, nematodes from coarser sediments, thriving in oxic conditions to relatively deep sediment layers (Vanaverbeke et al., 2004), may be more affected than species inhabiting fine sediments. Therefore, we investigate the response of different nematode communities from different sediment types to the same level of hypoxia in the overlying water. We test the hypothesis (H0) that duration of hypoxia does not affect the nematode community characteristics in the different sediment types.

2. Materials and methods

2.1. Study area

Three types of sediment were studied from the Belgian Part of the North Sea (BPNS). The shallow BPNS (average depth about 20 m, maximum depth ≤ 40 m) is characterised by a highly variable and complex topography, i.e. presence of sandbanks, and by different sediment types (Verfaillie et al., 2006). Sampling was carried out in three different stations with different oxygen regimes. St. 700 (12 m water depth, 51° 22' 618'' N, 3° 13' 148'' E), adjacent to the Zeebrugge harbor was characterized by muddy sediment with annual average oxygen penetration depth of 3.7 mm, St. 330 (23 m water depth, 51° 25' 984'' N, 2° 48' 502'' E), typically medium grained sediment with maximum oxygen penetration depth of 25 mm, and St. 115bis (25 m water depth, 51° 09' 151'' N, 2° 37' 029'' E), a station with fine sandy sediment and maximum oxygen penetration depth of 4.9 mm (Braeckman et al., 2014**;** Vanaverbeke and Braeckman, pers. Observ, Fig. 1).

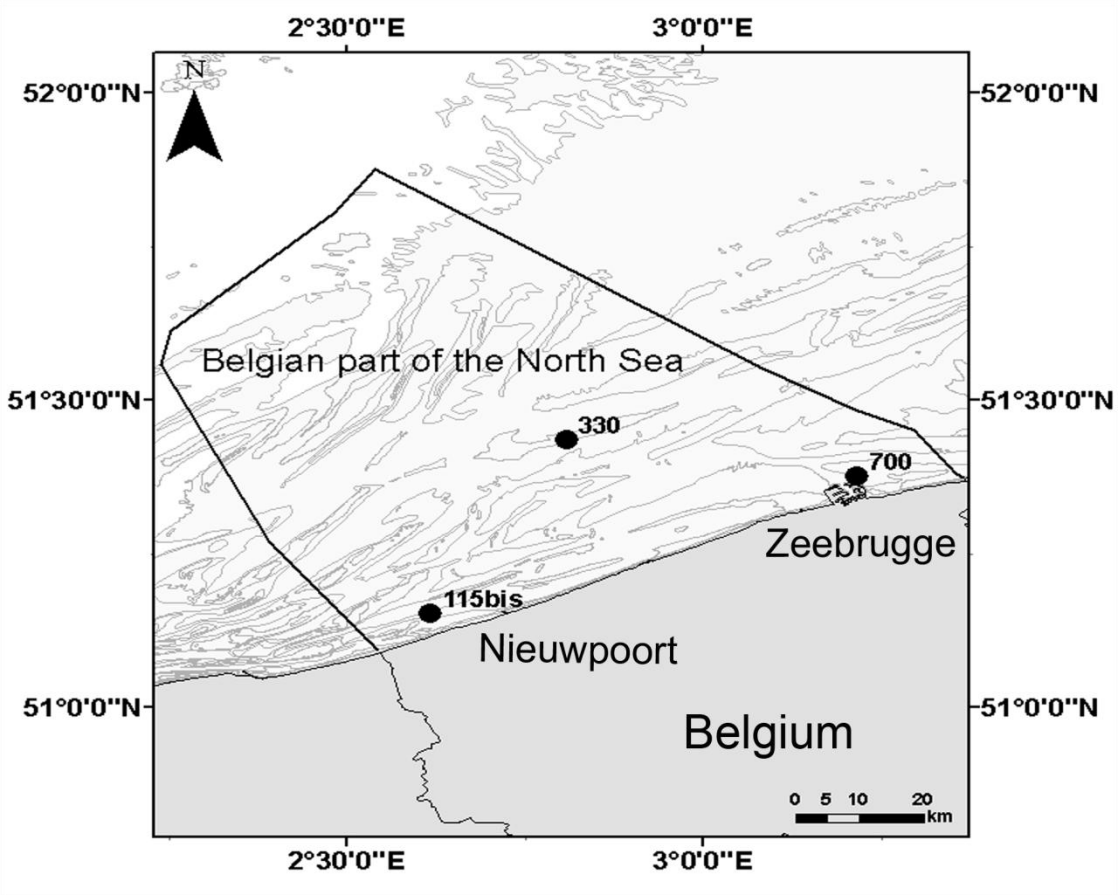


Figure 1. Map indicating sampling stations in the Belgian part of the North Sea.

2.2. Sampling strategy

Sediment was collected with a Reineck boxcorer from the *RV* Zeeleeuw in June 2011. The boxcorer was deployed five times in each station and six subsamples were collected with Perspex cores (surface 10 cm²). Three cores (from different deployments) were sliced on board in 1cm intervals down to 5 cm depth, for investigation of the *in situ* nematode communities characteristics (Vanaverbeke et al., 2003). These samples were fixed with a buffered 4% formaldehyde solution, and are further referred to as field control (FC). Similarly, another three cores (from different deployments) were sliced in 1 cm intervals down to 5 cm depth and stored onboard at -20 °C for grain size and

chlorophyll *a* analyses. These samples were transferred to -80 °C upon arrival in the lab and stored until further processing (Braeckman et al., 2011a). The remaining 72 cores (24 cores in every station) for treatments were kept at *in situ* temperature (16 °C) during the transport to the lab. In addition seawater with a salinity of 36 from each sampling site (1 m above the seabed) was collected with a 10 l Niskin bottle.

2.3. Experimental set-up and slicing

In the lab, the sediment cores were transported to a temperature controlled room (16 °C) and topped with seawater with a salinity of 36 from the corresponding sampling site and connected to air pumps overnight. For every station, cores were randomly allocated to four treatments. Oxic (control) and hypoxic treatments lasted 1 (Ox1, Hyp1) and 7 days (Ox7, Hyp7). The dissolved oxygen concentration in the overlying water of the control cores was kept constant (240.67 ± 4.15 µmol l^{-1} or 7.70 ± 0.09 mg l^{-1}) throughout the experiment by bubbling with air. Hypoxia (41.43 ± 3.78 µmol l^{-1} or 1.34 ± 0.09 mg l^{-1}; 17 % of saturation) was produced by bubbling with a mixture of nitrogen (97%) and oxygen (3%) from Air Liquide Company, Belgium (Steyaert et al., 2007). The cores were covered with perforated parafilm to avoid outgassing and evaporation and stored in the dark at 16 °C (Fig. 2). The oxygen concentration in the overlying water (Fig S.1) was regularly monitored with Unisense oxygen microsensors (type ox25). Vertical sediment oxygen profiles were measured at the end of the experiment in all cores using Unisense oxygen micro sensors (type ox25 in muddy and fine sandy sediment, and ox100 in medium sand) in vertical increments of 250 µm (Fig S.2 and S.3). After 1 and 7 days, three cores per treatment were sliced at 1 cm intervals down to 5 cm depth and each

slice was fixed in a buffered 4% formaldehyde solution (Vanaverbeke et al., 2003). Similarly, three cores were sliced at 1 cm intervals down to 5 cm depth and stored at -80 °C for grain size and chlorophyll *a* analyses.

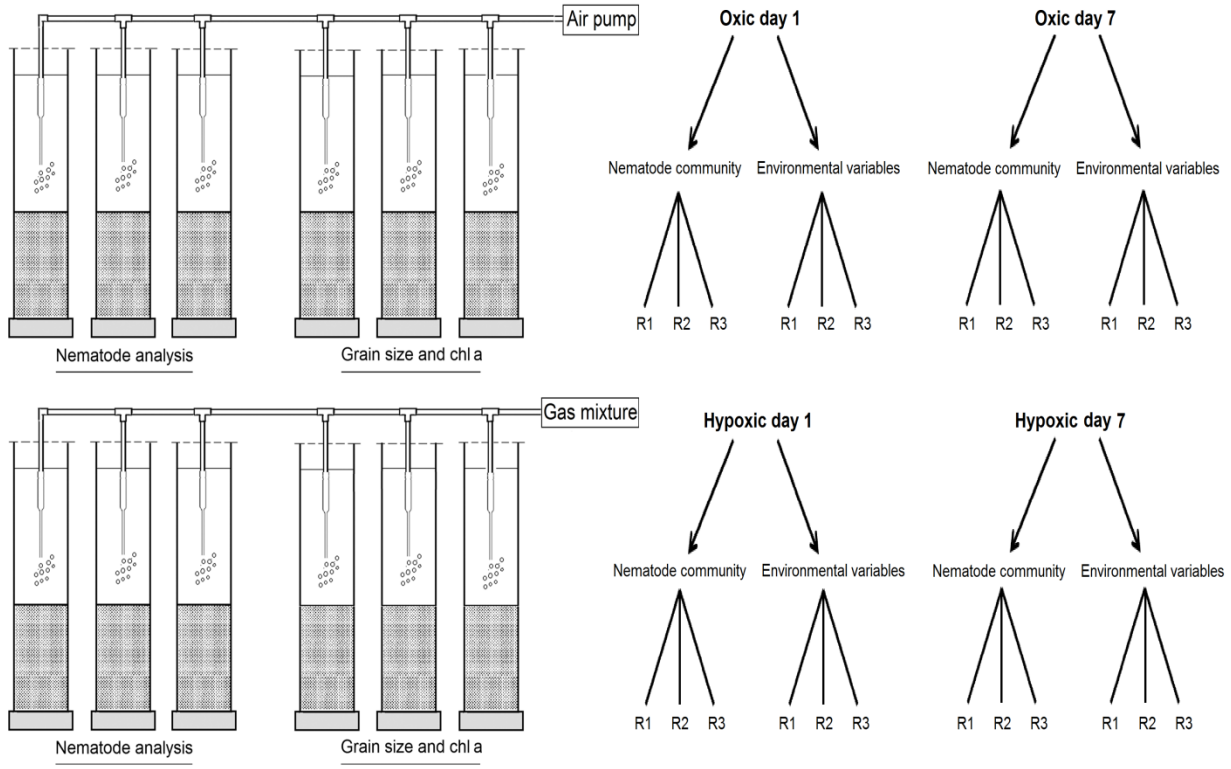


Figure 2. Schematic drawing of the experimental set up in every sediment types (cores were randomly allocated) for 1 and 7 days. Overlying water was continuously bubbled with air (Oxic treatment) or a gas mixture (Hypoxic treatment).

2.4. Laboratory processing

Metazoan meiobenthic organisms were extracted from the sediment of each slice by centrifugation with Ludox (Heip et al., 1985). Macrofauna was excluded by means of a 1 mm sieve and all animals retained on a 38 µm sieve (i.e. meiobenthos) were stained with Rose Bengal and counted. From two replicates, the first 120 nematodes were picked. When less than 120 individual were present, all nematodes were picked.

Nematodes were transferred to glycerine and mounted on slides for identification to species level (Vanaverbeke et al., 2004) according to the pictorial key of Platt and Warwick (1983, 1989), Warwick et al. (1998) and the NeMys online identification system (Vanaverbeke et al., 2015) and library of original species descriptions of the Marine Biology Research Group of Gent University.

Sediment subsamples were dried at 60 °C and grain size was determined with a Malvern Mastersizer using laser diffraction. Sediment types were defined according to the Wentworth scale (Buchanan, 1984). Chlorophyll *a* was extracted from the freeze-dried sediment with 10 ml of 90% acetone and its concentration (µg g^{-1} dw) in the supernatant was determined using HPLC (Gilson) analysis (Wright and Jeffrey, 1997).

2.5. Data analysis

To assess whether the sampled nematode communities were different, differences in total nematode density and diversity indices - Shannon-Wiener's (H', log e) and Pielou evenness (J) - among field control (FC) samples were compared using One-way Analysis of Variance (ANOVA). Homogeneity of variances was checked with the Levene's test. When overall significant differences were observed, a Tukey HSD test was used for pairwise comparisons. To test for differences in both vertical profiles of nematode densities (univariate) and community structure (multivariate) among FC samples, a fully crossed three factor design was analysed using PERMANOVA following Braeckman et al. (2011b). The design included station (St) and slice (Sl) as fixed factors and the random factor Replicate (Re) nested in station (St). A Euclidean distance and Bray-Curtis based resemblance matrix were used for univariate and

multivariate data, respectively. The interaction term St×Sl informs us about the difference in depth profiles of nematode densities or community structure among stations. Whenever significant differences were observed, pairwise tests of St within St×Sl were performed to investigate in which slice the stations differed. Due to the restricted number of possible permutations in pairwise tests, p-values were obtained from Monte Carlo samplings (Anderson and Robinson, 2003). A non-metrical Multidimensional scaling plot (MDS) based on Bray-Curtis similarity visualised the community structure. To investigate whether experimental handling of the cores affected the nematode communities, total density and diversity indices in FC and Ox1 cores were compared for every station, using a t-test. Differences in both vertical profile of nematode densities (univariate data) and community structure (multivariate data) were analysed using a fully crossed three factor design in PERMANOVA as described above. Furthermore, in all stations separates single factor treatment (TR) PERMANOVA analyses were run on 0–1cm slices for nematode communities structure (multivariate data).

Two-way ANOVA and subsequent Tukey HSD pairwise tests were used for assessing differences among nematode total density, diversity indices and maximum oxygen penetration depth in experimental conditions. In addition, the effect of short-term hypoxia on the vertical profile of total nematode densities (univariate), densities of the five dominant species (univariate) and on community structure (multivariate), a fully crossed four factor PERMANOVA was designed with Treatment (Tr), Day (Da), and slice (Sl) as fixed factors and random factor Replicate (Re) nested in Treatment (Tr). Whenever significant interaction terms were present, pairwise tests of Tr within

Tr×Da×Sl were performed. Furthermore, two factor (Day, TR) PERMANOVA analyses were run on 0–1 cm slices for nematode communities' structure (multivariate data) for the indivitial stations. Homogeneity of multivariate dispersion ('variance') was tested with PERMDISP for any of the significant terms in Permanova analyses. Non significant PERMDISP results indicate a significant PERMANOVA to be a difference due to location. All multivariate analyses were executed using untransformed (to contribute common species to the similarity) and 4^{th} root transformed data (to reduce the importance of dominant species to the similarity). Diversity indices (Evenness and Shannon-Wiener's) were calculated in PRIMER v6 with PERMANOVA+ add-on. PERMANOVA analyses were performed using this software as well. The remaining analyses and graphs were performed and drawn with the freely available R 2.14.2 software (http://www.r-project.org). All results are expressed as mean ± standard error.

3. Results

3.1. Environmental variables

Sediments from the three stations were strongly different. St. 115bis consisted of fine sand with 13.91% of mud. St. 330 consisted of medium sand devoid of mud. Finally St. 700 had coarse silt sediment with the highest mud fraction (72.35%). The chlorophyll *a* content was highest in St. 700, much lower in St. 115bis and very low in St. 330 (Table 1).

Table 1. Mean sediment variable measured in field control.

Station	Clay-silt (%)	Median grain size (µm)	Chlorophyll *a* (µg g^{-1} dw)
St. 700: Coarse silt	72.35±9.23	41.62±17.90	11.47±7.44
St. 115bis: Fine sand	13.91±12.84	165.90±20.07	1.82±1.86
St. 330: Medium sand	0.00±0.00	350.89±42.48	0.07±0.03

3.2. Field controls

Nematode densities ranged between 2421–5192 ind. 10 cm^{-2} in fine sand, between 423–885 ind. 10 cm^{-2} in medium sand and between 75–233 ind. 10 cm^{-2} in coarse silt sediment. Significant differences in total nematode densities ($F_{2,6}$ = 18.15, P = 0.001), Shannon diversity ($F_{2,3}$ = 24.42, P = 0.013), and evenness ($F_{2,3}$ = 83.91, P = 0.002) were observed among stations. Total density was significantly higher in the fine sand station compared to the other stations (Tukey HSD, $p < 0.05$). Shannon diversity (H') was significantly highest in the medium sand station, followed by the fine sand and the coarse silt station (Tukey HSD, $p < 0.05$). Evenness (J) was significantly higher in the medium sand station compared to the coarse silt station (Tukey HSD, $p < 0.05$) while no differences were found between the other sediment types (Tukey HSD, $p > 0.05$) (Fig. 3).

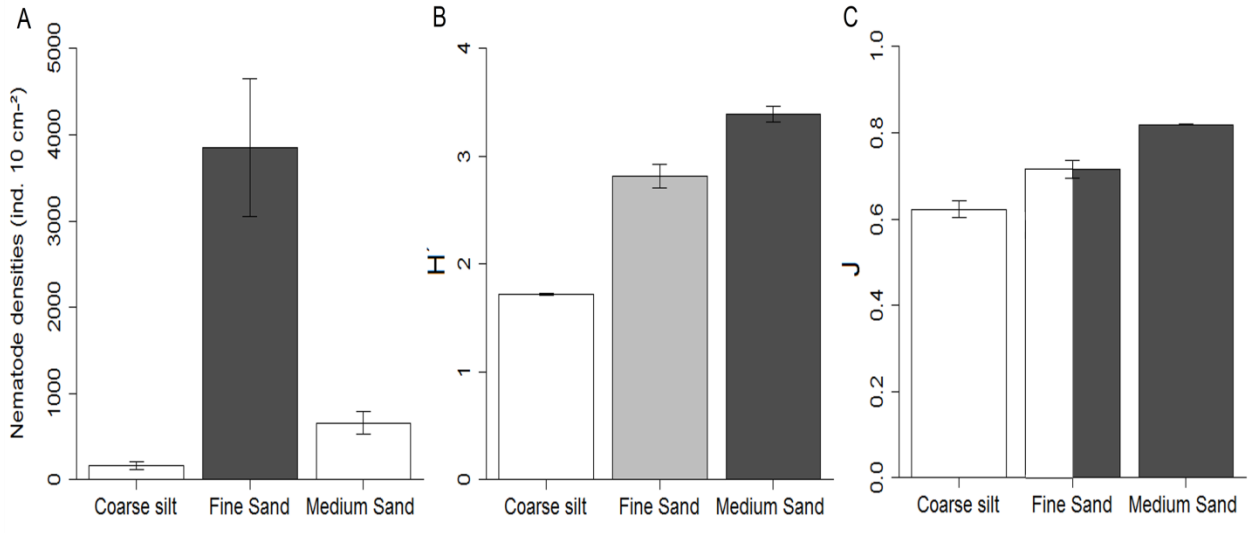


Figure 3. Total nematode densities (A, mean±SE, n=3), Shannon diversity (B) and evenness (C) in field control samples (mean±SE, n=2). Black, grey and white bars indicate the highest, middle and the lowest values. Same colour means no significant differences. Double colour bar indicates no significantly different from the other sediment types.

The vertical profiles of the nematode densities were significantly different among all sediment types. Highest densities per slice were generally observed in fine sand while lowest values were always found in coarse silt sediment (Table 2, Fig. 4). The PERMDISP test was not significant (F = 2.44, P (perm) = 0.651).

Table 2. Main and pairwise test results from PERMANOVA analysis for differences in nematode density and community structure among field controls. P (Per) = permutation, P (MC) = Monte Carlo, "*" P-values obtained from Monte-Carlo test.

	Vertical profile				Community structure			
	df	MS	Pseudo-F	P (Per)	df	MS	Pseudo-F	P (Per)
Station	2	1798.50	44.63	0.004*	2	19547.00	4.90	0.000*
Slice	4	84.98	3.57	0.023	4	2750.60	0.81	0.789
Re(St)	6	40.29	1.69	0.159	3	3985.50	1.18	0.242
Station × Slice	8	65.54	2.75	**0.027**	8	2774.10	0.82	0.865

Pairwise	115bis-330		115bis-700		330-700	
Slice (cm)	t	P (MC)	t	P (MC)	t	P (MC)
0–1	2.90	0.043	4.66	0.009	4.18	0.012
1–2	2.68	0.057	3.46	0.028	2.05	0.110
2–3	1.88	0.133	2.81	0.048	1.20	0.299
3–4	3.87	0.018	5.48	0.005	2.42	0.075
4–5	36.7	0.000	13.50	0.000	4.89	0.007

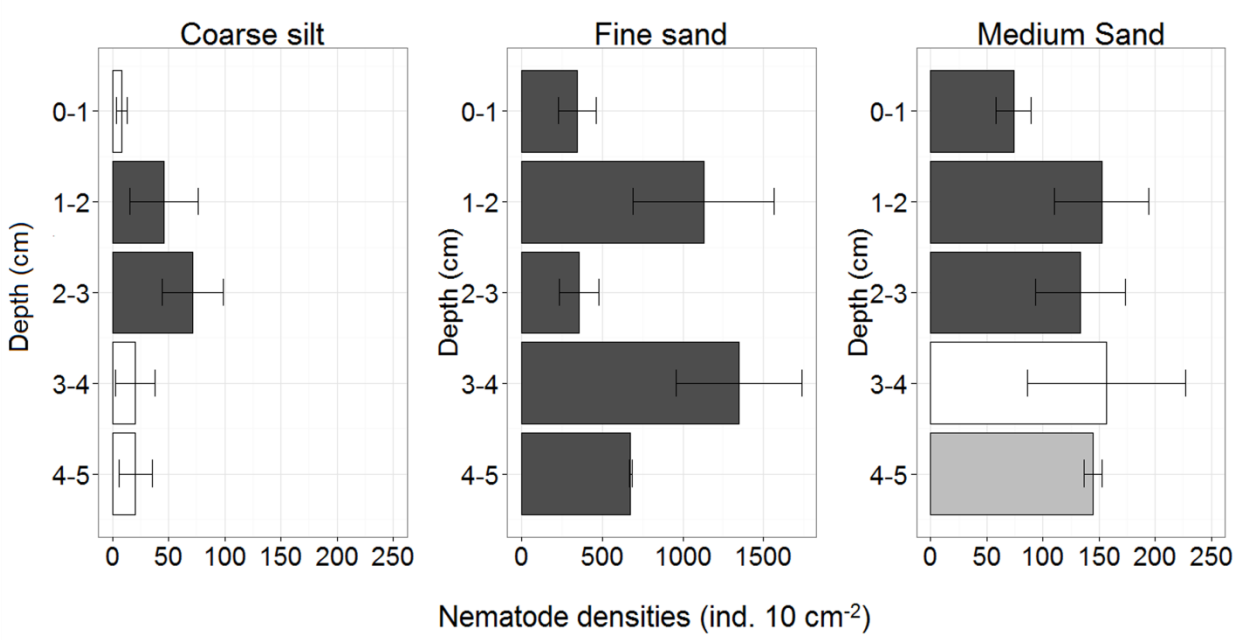


Figure 4. Vertical density profiles in field control (mean±SE, n=3). Darker colours reveal significantly higher densities in a slice compared to lighter colours in that slice in the other sediment types. Same colour means no significant differences. Gray coloured bars indicate no significant differences from the other sediment types. Note different scaling of x-axes.

Nematode community structure was significantly different among different sediment types (Table 2, Fig. 5) but no differences were observed among different sediment slices for the same sediment type or for the interaction term St×Sl (untransformed and 4th root transformed data). In the fine sand station, *Sabatieria pulchra* (Schneider, 1906), *S. celtica* (Southern, 1914) *and S. punctata* (Kreis, 1924) (50.6%) and *Microlaimus conothelis* (Lorenzen, 1973) (4.9%) were the most dominant species. *S.*

pulchra (Schneider, 1906)*, S. celtica and S. punctata* (80.2%) were dominant in the coarse silt station as well, followed by *Ascolaimus elongatus* (Bütschli, 1874) (6.6%). Finally, *Paracyatholaimoides asymmetricus* (Boucher, 1975) (11.3%) and *Rhynchonema megamphida* (Boucher, 1974) (6.4%) were the dominant species in the medium sand station. The PERMDISP test was not significant (F = 3.28, P (perm) = 0.078), revealing that difference in community structure was due to differences between sampling stations.

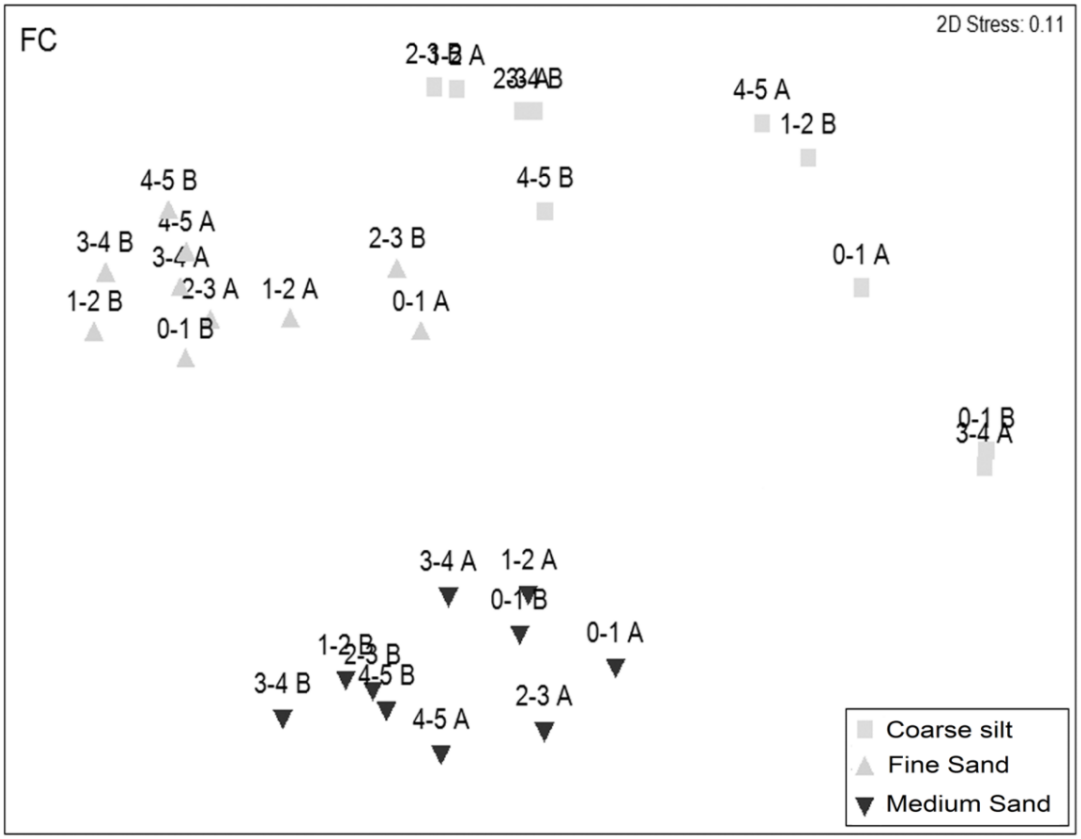


Figure 5. MDS plot based on Bray-Curtis similarity on total nematode density data in FC samples.

3.3. Effect of transport and experimental setup on nematode communities

Neither total nematode densities nor diversity indices were significantly affected by the transport to the lab and maintaining them in the experimental oxic settings for 1 day (All t-tests: $p > 0.05$). Apart from significantly higher density in the deepest layer (4-5 cm) in fine sand sediment in the Ox1 treatment, vertical density profiles and community structure (untransformed and 4^{th} root transformed data) in the experimental situation were not significantly different from the FC situation. A non significant dispersion effect was detected for vertical density profiles in fine sand sediment (PERMDISP, F = 2.13, P (perm) = 0.750). In addition, the community structure in the upper cm layer of all sediment types were not significantly affected by the transport to the lab (Table 3, Tables S.1 and S.3, Fig. S4).

Table 3. Main and significantly different pairwise test results from PERMANOVA analysis for differences in nematode density and community structure between field control and Ox1 in all stations. P (Per) = permutation, P (MC) = Monte Carlo, "*" P-values obtained from Monte-Carlo test.

		Vertical profile				**Community structure**		
	df	MS	Pseudo-F	P (Per)	df	MS	Pseudo-F	P (Per)
Coarse silt								
Treatment	1	3060.30	0.76	0.425*	1	2674.30	0.67	0.667*
Slice	4	3891.40	4.24	0.014	4	3793.90	0.91	0.572
Re(Tr)	4	3979.20	4.34	0.015	2	3947.60	0.95	0.500
Treatment × Slice	4	309.22	0.33	0.840	4	3065.10	0.74	0.779
Fine sand								
Treatment	1	970200.00	3.42	0.137*	1	4996.40	1.46	0.260*
Slice	4	271680.00	2.26	0.107	4	2218.70	0.84	0.691
Re(Tr)	4	283140.00	2.36	0.098	2	3405.40	1.29	0.228
Treatment × Slice	4	454280.00	3.79	**0.027**	4	3485.30	1.32	0.214

Pairwise test								
Slice (cm)		t	P (MC)					
4–5		7.87	0.001					
Medium sand								
Treatment	1	8534.50	0.26	0.634*	1	11909.00	2.24	0.101*
Slice	4	2387.00	0.64	0.636	4	2945.50	1.18	0.224
Re(Tr)	4	31666.00	8.52	0.000	2	5302.60	2.13	0.013
Treatment × Slice	4	5079.20	1.36	0.292	4	2914.80	1.17	0.249

3.4. Effects of hypoxia

During the experiment, average oxygen concentrations in the overlying water were 239.72 ± 0.53 and 37.82 ± 1.68 µmol l^{-1} at day 1 and 240.67 ± 4.15 and 41.43 ± 3.78 µmol l^{-1} after 7 days (which equals on average 7.70 and 1.34 mg l^{-1}) in oxic and hypoxic treatments, respectively. Hypoxia in the overlying water did not affect the oxygen penetration depth in the medium sand station while oxygen penetration depth was deepest in the 7 day oxic (Ox7 vs, Hyp 7) treatment in the other stations, with a maximum of -7.25 mm measured in coarse silt (Table 4, Fig. 6).

Table 4. Results from two-way ANOVA and results from the significant Tukey HSD test of maximum oxygen penetration depth in all stations.

	Coarse silt		**Fine sand**		**Medium sand**	
	F	P	F	P	F	F
Treatment	6.66	0.000	100.99	0.000	14.77	14.77
Day	35.15	0.000	14.93	0.006	2.50	2.50
Treatment × Day	20.55	**0.001**	29.16	**0.001**	0.14	0.14
Pairwise test						
Ox 1–Hyp 1		0.176		0.085		
Ox 7–Hyp 7		**0.000**		**0.000**		

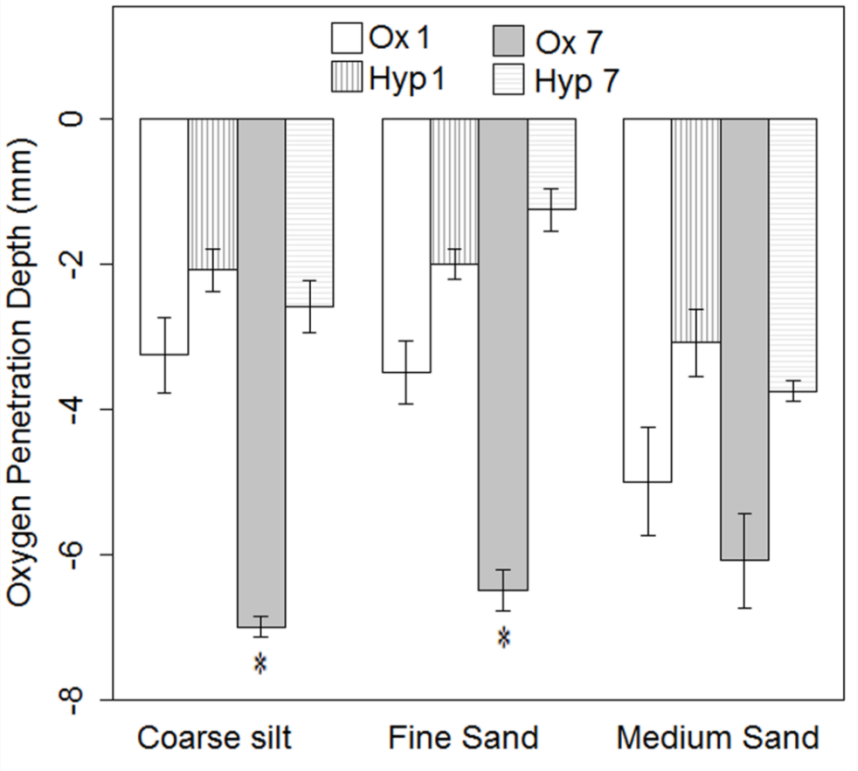


Figure 6. Maximum oxygen penetration depth in the different treatments (mean±SE, n=3). Significantly highest values are marked with "*".

Both total nematode densities and Shannon diversity (H') were unaffected by the experimentally induced water column hypoxia in all sediment types. A significant difference was observed for evenness (J) in the fine sand station between Ox1 and Ox7 treatments (Table 5, Fig. 7).

Table 5. Results from two-way ANOVA and results from the significant Tukey HSD test on total density, Shannon and evenness in all stations.

	Coarse silt		**Fine sand**		**Medium sand**	
Density	F	P	F	P	F	P
Treatment	0.90	0.368	0.14	0.715	1.60	0.240
Day	1.07	0.330	3.40	0.10	0.28	0.608
Treatment × Day	0.46	0.514	0.005	0.94	0.37	0.852

Shannon						
Treatment	0.02	0.885	0.85	0.408	0.04	0.845
Day	0.17	0.694	0.50	0.516	1.15	0.342
Treatment × Day	0.15	0.713	0.02	0.887	3.98	0.11
Evenness						
Treatment	0.07	0.801	0.04	0.841	2.97	0.159
Day	0.02	0.965	47.36	0.002	1.26	0.324
Treatment × Day	0.01	0.920	45.60	**0.002**	6.28	0.066
Pairwise test						
Ox 1–Ox 7		0.002				

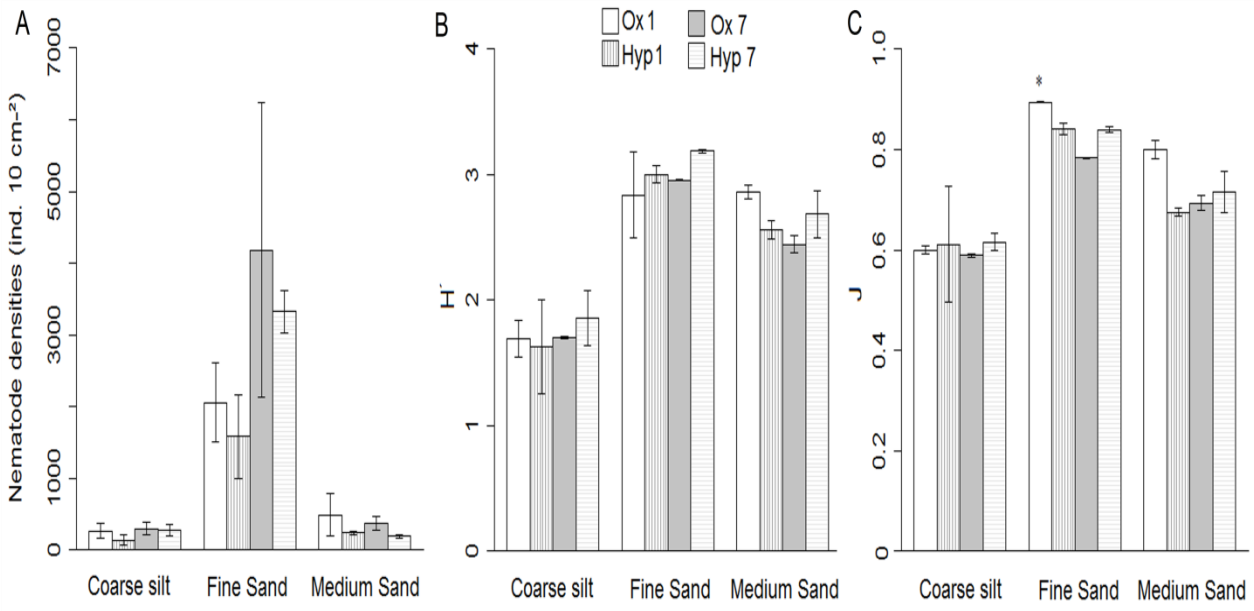


Figure 7. Total nematode densities (A, mean±SE, n=3), shannon diversity (B) and evenness (C) (mean±SE, n=2) in oxic and hypoxic treatments (Day 1 and 7) at different sediment types. Significantly highest value is marked with "*".

The vertical profiles of the nematode densities were not affected by hypoxia in medium sand and coarse silt stations. In fine sand sediment, significantly higher density was observed in the hypoxic treatment in comparison with oxic in day 7 (Table 6, Fig. 8) but PERMDISP test was not significant (PERMDISP, F = 4.26, P (perm) = 0.068). In terms of nematode community structure (untransformed and 4^{th} root transformed data), there were no significant differences associated with treatments, time or any interaction effects (Table 6, Tables S.2). When analysing the community structure in the upper cm separately, treatment, time and their interaction was also not significant (Tables S.4, Fig. S5). When examining the vertical distribution of the five dominant nematodes in each station, only density of *Sabatieria breviseta* at 0–1 cm from coarse silt sediment showed significant differences at day 7 (Table 7).

Table 6. Main and pairwise test results from PERMANOVA analysis for differences in nematode density and community structure among treatments in all stations. P (Per) = permutation, P (MC) = Monte Carlo, "*" P-values obtained from Monte-Carlo test.

	Vertical profile				Community structure			
	df	MS	Pseudo-F	P (Per)	df	MS	Pseudo-F	P (Per)
Coarse silt								
Treatment	1	3969.10	0.95	0.374*	1	6079.30	1.17	0.372*
Day	1	6283.30	1.61	0.280	1	6073.10	1.42	0.299
Slice	4	11069.00	5.12	0.007	4	6865.20	2.00	0.017
Re(Tr)	4	4137.40	4.16	0.016	2	5162.40	2.17	0.031
Treatment × Day	1	1251.30	0.32	0.599	1	3943.80	0.92	0.523
Treatment × Slice	4	788.19	0.36	0.827	4	3510.60	1.02	0.455
Day × Slice	4	1251.00	1.25	0.327	4	4141.60	1.74	0.037
Day × Re(Tr)	4	3893.70	3.91	0.018	2	4271.70	1.80	0.069
Slice × Re(Tr)	16	2159.90	2.17	0.056	8	3420.30	1.44	0.064
Treatment × Day × Slice	4	1966.50	1.97	0.150	4	3119.70	1.31	0.166

Fine sand								
Treatment	1	401470.00	0.41	0.555*	1	7217.60	1.38	0.299*
Day	1	2622500.00	7.40	0.053	1	10792.00	3.46	0.092
Slice	4	883420.00	9.15	0.000	4	5427.40	2.78	0.007
Re(Tr)	4	965870.00	9.91	0.000	2	5218.20	2.075	0.057
Treatment × Day	1	214320.00	0.60	0.478	1	2746.60	0.88	0.525
Treatment × Slice	4	248540.00	2.57	0.078	4	2385.60	1.22	0.257
Day × Slice	4	307690.00	3.15	0.042	4	2583.60	1.02	0.440
Day × Re(Tr)	4	354110.00	3.635	0.024	2	3117.60	1.23	0.282
Slice × Re(Tr)	16	96455.00	0.99	0.508	8	1947.90	0.77	0.825
Treatment × Day × Slice	4	439510.00	4.51	**0.010**	4	2153.00	0.85	0.659
Pairwise test		t	P (MC)					
Ox7– Hyp7, 0–1 cm		3.45	0.025					
Medium sand								
Treatment	1	30150.00	2.77	0.169*	1	2400.70	0.65	0.719*
Day	1	3300.40	0.17	0.700	1	3429.60	1.08	0.412
Slice	4	6752.40	4.96	0.008	4	3719.10	1.23	0.238
Re(Tr)	4	10860.00	7.0397	0.001	2	3674.90	1.75	0.058
Treatment × Day	1	534.02	2.79	0.872	1	8435.70	2.65	0.145
Treatment × Slice	4	3586.10	2.63	0.075	4	2396.80	0.79	0.824
Day × Slice	4	652.37	0.42	0.791	4	2275.90	1.08	0.370
Day × Re(Tr)	4	19138.00	12.40	0.000	2	3184.90	1.51	0.122
Slice × Re(Tr)	16	1359.80	0.88	0.595	8	3024.50	1.44	0.041
Treatment × Day × Slice	4	224.39	0.14	0.961	4	3066.70	1.46	0.095

Table 7. PERMANOVA results for the 5 most abundant nematode species in all station. P (Per) = permutation.

		Treatment × Day × Slice		
	df	MS	Pseudo-F	P (per)
Coarse silt				
Ascolaimus elongatus	4	5.89	0.15	0.957
Oncholaimellus calvadosicus (de Man, 1980)	4	29.03	1.39	0.336
S. breviseta	4	344.53	9.90	**0.006**
S. elongata	4	8.83	1.17	0.398
S. punctata	4	11.11	0.15	0.956
Pairwise test		t	P (MC)	
Ox7– Hyp7, 0–1 cm		7.34	0.016	
Fine sand				
Paramonhystera longicaudata (Steiner, 1916)	4	69.21	2.16	0.161
Sabatieria breviseta	4	37.96	0.89	0.518
S. celtica	4	59.91	0.31	0.855
S. punctata	4	48.93	0.32	0.853
Microlaimus conothelis	4	35.08	1.71	0.240
Medium sand				
Epsilonema serrulatum (Steiner, 1927)	4	1.65	0.72	0.608
Paracyatholaimoides asymmetricus	4	9.31	2.46	0.141
Prochromadorella attenuata (Gerlach, 1953)	4	28.76	1.56	0.279
Rhynchonema megamphida	4	3.33	0.39	0.810
Xyala riemanni (Boucher and Helléouët, 1977)	4	2.34	1.37	0.335

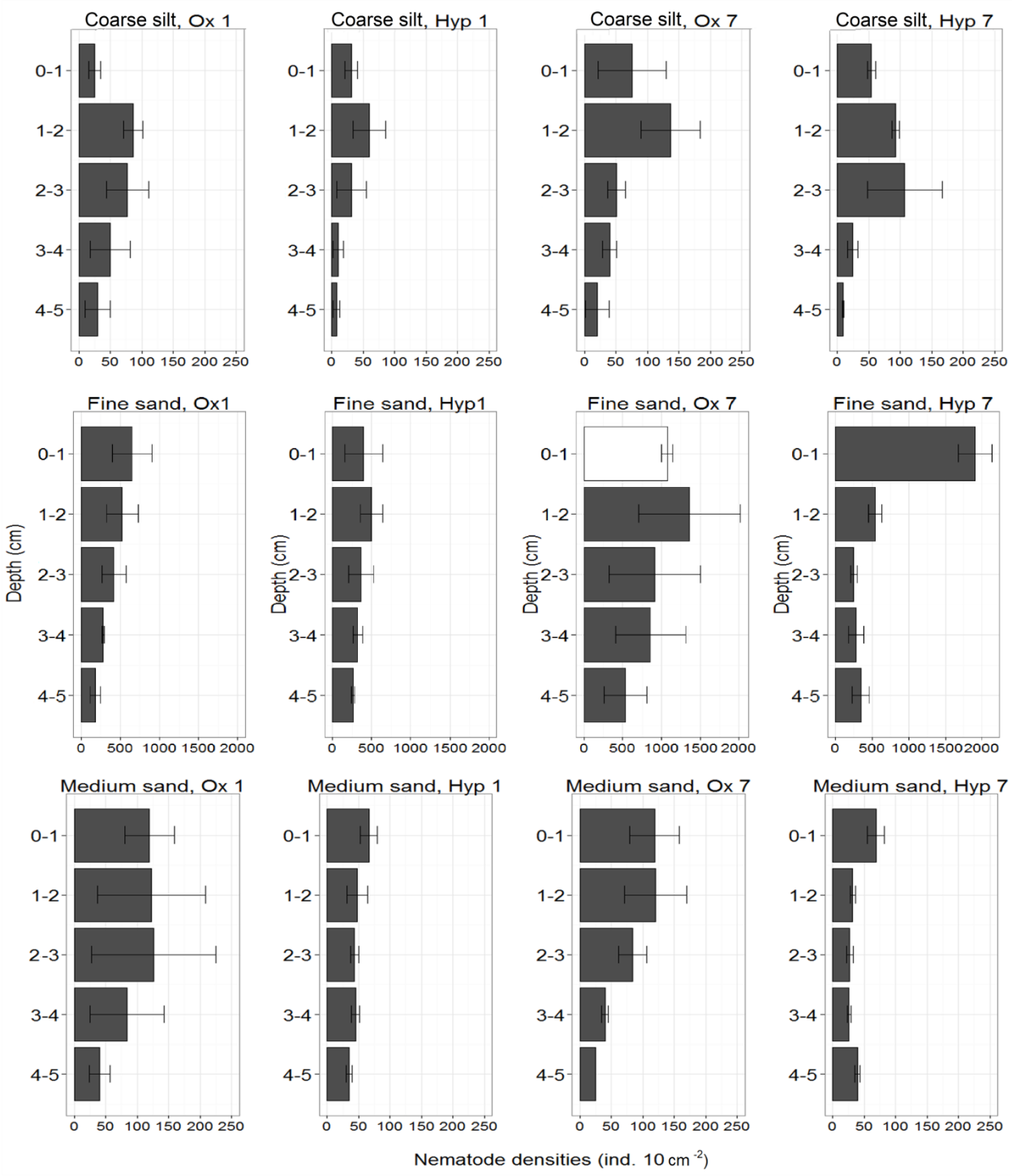


Figure 8. Vertical density profiles (mean±SE, n=3) in oxic and hypoxic treatments (day 1 and 7). White bar indicate lower significant density in oxic day 7 in comparison with hypoxic day 7. Note different scaling of x-axes.

4. Discussion

Our results indicate that the experimental handling of sediments had no adverse effects on the nematode communities. Detailed changes in the vertical distribution of nematode communities or sensitive species in the upper cm layer induced by changes in oxygenation on the mm-scale cannot be assessed by our sampling design, as we sliced the sediment per cm. Therefore, we tested whether the community structure of the nematode communities from the upper cm only were affected. Our analyses showed that this was not the case. Hence, we report on the general response of sediment-inhabiting nematodes to water-column hypoxia.

Nematode vertical distribution in marine sediments is related to several factors such as sediment oxygen concentration (Hendelberg and Jensen 1993), food availability in the sediment (Franco et al. 2008a) and macrofaunal activity and disturbance (Braeckman et al., 2011b). In North Sea sediments significant subsurface peaks have been naturally observed before (e.g. Steyaert, et al., 1999, 2003; Vanaverbeke et al., 2004; Braeckman et al., 2011b). In the present study, the low natural densities in the upper cm in course silt and fine sand stations could be related to distribution of nematodes which are resistant to lack of oxygen like *Sabatieria* (dominant genera in both stations) at that depth. This downwards shift is likely to avoid disturbance and competition with some macrofauna at the surface, while they might have benefited from the macrofauna faecal pellets deposited in the subsurface (Braeckman et al., 2011b).

Our results also show that, under laboratory conditions, exposure to short-term hypoxia had no effects on density, species diversity, community structure and vertical distribution of nematodes, independent of the sediment type. Even when hypoxia was

imposed to the water overlying sediments that are oxygenated throughout the year (St. 330- medium sand; Vanaverbeke et al., 2003), nematode communities were not adversely affected.

In the natural environment, oxygen concentration in the sediment is a result of the interplay between oxygen consumption (mineralization process) and diffusion, and macrofaunal activity including bioturbation and bio-irrigation (Cai and Sayles, 1996; Glud, 2008). In sediments with higher median grain size and increased permeability, advective flows through the sediments cause a deep oxygen penetration (de Beer et al., 2005). Thus, we expected a maximum oxygen penetration depth in the medium sand treatments (Vanaverbeke et al., 2011) when compared to the fine sand and the coarse silt sediment. However, oxygen profiles measured in the Ox1 treatments did not fully match the expectations as oxygen penetration depths in sediments from the medium sandy station were not markedly deeper. A possible explanation may be the lack of advective flows in our experiment. Previous incubations of medium sand (St. 330) sediments in benthic stirring discs, for example, resulted in deeper (max. 25 mm) penetration depths for oxygen (Vanaverbeke and Braeckman, pers. observ.). The significantly higher oxygen penetration depths in the oxic treatments of both other stations at day 7 are probably due to the fact that our oxygen inlet in the cores was close to the sediment-water interface, resulting in a deeper oxygen penetration compared to the onset of the experiment. Comparing the oxygen penetration depths of the hypoxic treatments of these stations with those observed in the control treatments at day 1, reveals an upward migration of 1–2 mm which is however, not significant.

The overall low impact of short-term hypoxia on nematode total density, diversity, vertical profiles and community structure (dominant and rare species) in all sediment types did not reflect our expectations, i.e. increased nematode mortality, an associated change in diversity and community structure and an escape from oxygen-stressed layers (Steyaert et al., 2007; Arroyo et al., 2012) even by nematodes from the upper cm, which are more sensitive to overlaying water oxygen stress (Jensen, 1984). Two explanations are possible. First, we rather believe that different experimental set-ups have introduced additional stressors: Arroyo et al. (2012), for example, describe the development of sulphidic conditions, the accumulation of ammonia and the presence of predators as possible additional factors acting in combination with hypoxia, as possible reasons for decreases in meiofaunal densities. Although not explicitly stated by Steyaert et al. (2007), where intertidal sediments were incubated with hypoxic water for 2 weeks in airtight-sealed experimental units, similar additional affects (development of sulphidic conditions, accumulation of ammonia) might have negatively affected the nematode communities. Second, apart from the possible build-up of toxic substances in stagnant water and sediments in experimental units (Vaquer-Sunyer and Duarte, 2010; Arroyo et al., 2012; Riedel et al., 2012), additional effects on the intertidal nematodes can be introduced by the lack of the daily exposed period. In intertidal areas, tidal fluctuations (inflow and outflow at high and low tide) can generate an advective current in the sediment which plays a major role in the oxygenation process of intertidal zones (Precht et al., 2004) so that oxygen penetration depth is deeper (3 mm in inundated to more than 7 mm in exposed sediment) at atmosphere exposed sediments (Brotas et al., 1990). This sediment ventilation is also related to surface topography and flow velocity.

With increasing flow velocity (10 cm s^{-1}) oxygen penetration depth can reach even 40 mm (Ziebis et al., 1996). Prolonged incubation (2 weeks) of sediments in closed; continuously inundated intertidal sediments might result in the creation of a very harsh environment for the nematodes, and the subsequently higher mortality rates.

Apart from methodological considerations, differences in environmental conditions may be one explanation for different impacts on the nematode community (Wetzel et al., 2001; Tahseen, 2012). Annual average of maximum oxygen penetration depth in medium sand (St. 115bis, max. 4.9 mm, Vanaverbeke and Braeckman, unpublished data) and coarse silt (St. 700, max. 3.7 mm; Braeckman et al., 2014) are limited to the first 5 mm. These two stations are located close to the sea shore and probably their low oxygen penetration depth could be related to sedimentation of phytodetritus or especially in station 700 as a result of pollution originating from the harbor. Many nematode species can persist over extended periods in deeper anoxic subtidal sediments (Steyaert et al., 1999; Franco et al., 2008a; Braeckman et al., 2011a,b, this study), probably to escape competition with and disturbance by larger macrofaunal organisms at the sediment-water interface (Braeckman et al., 2011b). Hence, nematodes should be adapted to the prevailing oxygen depleted circumstances there. Adaptation mechanisms are probably related to exposure period, phylogenetic constraints and lifestyle. Low oxygen demand, a high surface to volume ratio and anaerobic metabolism enable some species to survive in hypoxia or anoxia for extended times (Wetzel et al., 2001). In general, nematodes living in deeper sediment layers (anoxic layer) were significantly more slender than their oxybiotic, surface-dwelling congeners (Tahseen, 2012). Soetaert et al. (2002) showed that the increased

length/width ratio at deeper sediment horizons was caused by an increase in length (rather than a decrease in width), which allows to bridge the gap between oxic and anoxic spots in the sediment, and allows for faster migration between oxygenated and less-oxygenated layers. In the otherwise fully oxygenated sediments of St. 330, such vertical pattern in nematode length/width ratios was absent (Vanaverbeke et al., 2004). However, nematode communities from medium sand (St. 330) were not negatively affected in our experiment, suggesting that other factors can contribute to the resistance to short-term hypoxic events.

Alternating between aerobic and anaerobic metabolism has been reported in some nematodes (Tahseen, 2012). Ott and Schiemer (1973) studied 24 nematode species and showed that respiration rates (metabolism) of nematodes inhabiting permanently anoxic sediments were significantly lower than nematodes in oxic environments. In addition, Braeckman et al. (2013) observed decreased nematode (*Enoploides longispiculosus*) respiration rates in hypoxic environments, suggesting that this species can decrease its metabolism when oxygen is limiting, which may explain the lack of negative response to short-term hypoxia of the nematodes inhabiting the fully oxygenized coarse sediments. This would suggest that the capacity of nematodes to decrease their metabolism when stressed is a widespread life-history trait, and merits further research.

Finally, our results showed that the vertical density profiles of dominant nematode species were not affected by short-term hypoxia (except for one species in the coarse silt station). In our experiment, the presence of *Sabatieria* in all sediment types as a dominant genus can also explain the lack of significant response of the nematode

communities to hypoxia since this genus is well adapted to survive under short and long periods of hypoxia and inhabits all types of sediment (Modig and Olafsson, 1998; Wetzel et al., 2002; Steyaert et al., 2007; Levin et al., 2009).

While short-term hypoxia has no effect on our subtidal nematode communities, we do believe that long-term patterns in oxygenation in the natural environment do affect the diversity, density and community structure of nematode communities. This is reflected in the significant differences we encountered in the field control samples of the different sediment types. Highest diversity in coarse sediments, lowest densities and diversity in finest sediments and high density and intermediate diversity in medium sediments was documented before and related to food availability and oxygenation (Vanaverbeke et al., 2011). High oxygen concentrations allow for a high diversity, whilst low food availability limits nematode densities (St. 330). When oxygen stress becomes too high during a long period, only well-adapted species survive in low quantities (St. 700), while intermediate situations (St. 115 bis) result in high nematode densities with intermediate diversity.

5. Conclusions

In conclusion, we show that short-term hypoxia does not lead to negative effects on nematode communities from subtidal sediments. This holds for different nematode communities, even for those communities inhabiting well-oxygenated sediments. These findings have important consequences for the use of subtidal nematode communities in monitoring programmes. Different aspects of nematode communities indeed reflect

long-term oxygen stress, but our results do not support the use of nematode communities to detect short-term hypoxic events.

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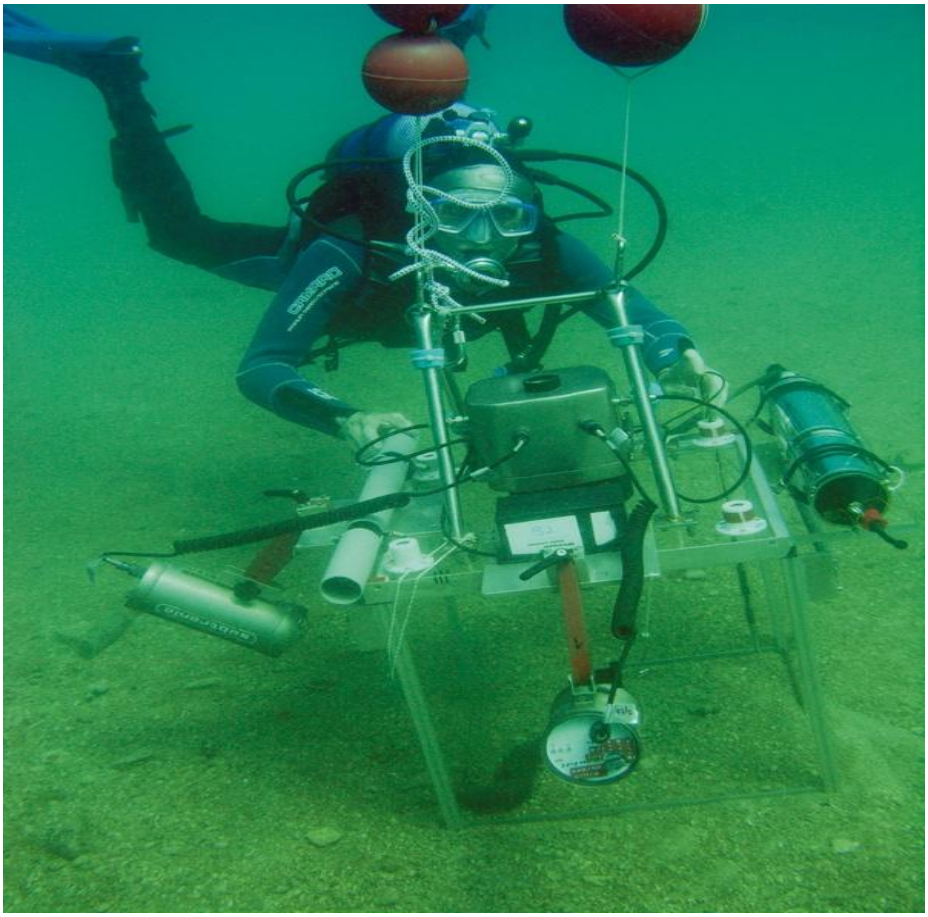
Chapter 3

Patterns in nematode community during and after experimentally induced anoxia in the northern Adriatic Sea



Modified from the following publication:

Mehrshad Taheri, Mateja Grego, Bettina Riedel, Magda Vincx and Jan Vanaverbeke. 2015. Patterns in nematode community during and after experimentally induced anoxia in the northern Adriatic Sea. Marine Environmental Research, 110: 110-123.

Abstract

The effect of short and long-term induced anoxia on a benthic nematode community and its potential for recovery after reoxygenation were investigated in an *in situ* experiment on a silty-sand bottom in the Gulf of Trieste, the northern Adriatic Sea. Anoxia was created artificially by three underwater benthic Plexiglas chambers at a depth of 24 meter. Treatments lasted for 2, 23 and 307 days. Control samples (Normoxia) were taken on 3 (Normoxia 1) and 25 (Normoxia 2) August 2010 outside the chambers (4 to 5 m further). After opening the chambers, recovery cores were taken after 7 days (Anoxia 2D), 30 days (Anoxia 23D) and 90 days (Anoxia 307D).

Our results revealed that short-term anoxia (Anoxia 2D) did not affect nematode total density and diversity, community structure and their vertical distribution in the sediment. However, total and vertical nematode density, species richness and diversity decreased at 23 days and decreased further at 307 days anoxia. Some nematode species like *Metalinhomoeus effilatus, Paralinhomoeus caxinus* and *Terschellingia longicaudata* even survived in the 307 days anoxia treatment. Our results also demonstrated that nematode community exposed to 23 days anoxia did not recover after 30 days sediment reoxygenation but, a full recovery was observed after 90 days for nematode community exposed to 307 days of anoxia. However, this full recovery could be related to the small scale of impacted area as well.

Feeding type contribution (functional aspect) of the nematode community also changed in the anoxia treatments and during the recovery process. This change was most drastic in the Anoxia 23D and 307D treatments. In both Normoxia and Anoxia 2D treatments, selective deposit feeders (1A), non-selective deposit feeders (1B) and epistrate feeders

(2A) were observed in the dominant nematode community. Epistrate feeders disappeared in the Anoxia 23D treatment, epistrate and also selective deposit feeders did not belong to the dominant nematode species in the Anoxia 307D treatment. After the recovery process, epistrate feeders and selective deposit feeding nematodes reappeared again amongst the dominant nematode species after 30 and 90 days of recovery, respectively.

Keywords Marine nematodes, Anoxia, Recovery, Community structure, Gulf of Trieste, Adriatic Sea

1. Introduction

Hypoxia (oxygen concentration ≤ 2 mg l^{-1}) and anoxia (oxygen concentration = 0 mg l^{-1}) are serious problems in coastal waters (Diaz and Rosenberg, 2008), affecting biogeochemical processes in the sediment and adversely affecting benthic animal life (Middelburg and Levin, 2009; Vaquer-Sunyer and Duarte, 2010; Riedel et al., 2014). Dissolved oxygen depletion can cause mortality, reductions in total macrobenthic biomass (Sturdivant et al., 2013) and changes in behavioural reactions of macrofaunal organisms (Riedel et al., 2014). In general, meiofauna are more resistant than macrofauna to hypoxic and anoxic conditions (Levin et al., 1991; Van Colen et al., 2009). The response of marine benthic meiofauna to anoxia is species-specific and related to the duration of anoxia and the natural adaptations of the species to live in oxygen-stressed environments (Modig and Olafsson, 1998; Travizi, 2000; Steyaert et al., 2007). Copepods are the most sensitive meiofaunal group for oxygen stresses (Moodley et al., 1997; De Troch et al., 2013) whereas hard-shelled foraminifera are among the most resistant group, even to prolonged periods of anoxia in coastal sediments (Moodley et al., 1997; Langlet et al., 2014). Marine free-living nematodes, dominant within the meiofauna, prefer to live in oxygenated sediment (Wetzel et al., 2001; Steyaert et al., 2005). Earlier studies have shown that nematode total density and diversity, vertical distribution in the sediment and community composition are not affected by short-term (≤ 1 week) hypoxia (Taheri et al., 2014) or even anoxia (Guerriniet al., 1998; Steyaert et al., 2005). Mid-term anoxia (2 weeks) leads to increased mortality, changes in the nematode community structure and altered vertical distribution patterns of marine nematodes (Travizi, 2000; Steyaert et al., 2007). Stronger

effects were observed during longer-term oxygen stress (≥ 1 month). A one month anoxic event in the southwest Baltic Sea, for example, showed a negative effect on nematode density and species richness (Wetzel et al., 2002). Longer periods of hypoxia/anoxia can cause a decrease in nematode density and changes in the community composition (Moodley et al., 1997; Van Colen et al., 2009). Some hypoxia/anoxia studies have shown that nematode community distribution was more affected by macrofaunal activity (Moodley et al., 2000) or food quality (Cook et al., 2000) than oxygen availability in sediment. The contrasting results mentioned above could be related to species-specific responses in nematode communities to oxygen stress and exposure regime.

Recovery of most benthic groups from hypoxia/anoxia starts as soon as the dissolved oxygen concentrations in the water column reaches 3 mg l^{-1} again (Steckbauer et al., 2011). Some studies have shown that nematode communities recover faster than the other meiofaunal groups in the sediment column (Sherman and Coull, 1980; Colangelo and Ceccherelli, 1994; Travizi, 1998). The recolonization starts with the arrival of nematode species from surrounding, non-impacted environments by active swimming or migration through the sediment, as well as settling after passive movement through the water column, rather than by reproduction of local individuals that have survived the oxygen stress (Guerriniet al., 1998; Wetzel et al., 2001). Furthermore, the recovery of the nematode community is closely linked with the recovery of the local macrobentic community as well (Van Colen et al., 2009). Macrobenthic activities such as bioturbation and bio-irrigation transport food and oxygen from the surface sediment to deeper layers, creating a suitable habitat for nematodes (Braeckman et al., 2011b). However, the

recolonization patterns of the nematode community are species-specific, complex, and are do not follow a clear pattern (Guerriniet al., 1998; Wetzel et al., 2001, 2002). For example, one month after an anoxic event (duration 1 month), Wetzel et al. (2002) observed a nematode recolonization only in the first centimetre of the sediment, suggesting that the recovery process in deeper sediment layers requires a longer period of time. The recovery process depends on several biotic and abiotic factors (Wetzel et al., 2002; Van Colen et al., 2009). Therefore, *in situ* experiments provide an ideal tool to investigate both the effect of oxygen stress, and the recovery from that stress on benthic communities, when executed in well-chosen areas.

The Gulf of Trieste is a semi-enclosed, shallow marine basin with high riverine inputs of nutrients and organic matter in the northern part of the Adriatic Sea (Giani et al., 2012). Late-summer stratification of the water column can trigger the development of seasonal hypoxia and anoxia here (Malej and Malacic, 1995; Giani et al., 2012). While most studies investigating the effect of oxygen stress on marine nematodes have been conducted in the lab, and involved nematode communities mainly living in mostly oxygenated sediments (i.e. Steyaert et al., 2005; Steyaert et al., 2007; Taheri et al., 2014), information from *in situ* experiments, taking into account communities adapted to recurrent anoxic events are lacking. Meiofauna communities from such environments are more tolerant and resilient to low oxygen conditions (Travizi, 1998; Wetzel et al., 2001), but detailed investigations on the long-term effects of oxygen stress and subsequent recovery are lacking. Hence, the Gulf of Trieste is an ideal setting for investigating the effect of anoxia on and recovery of marine benthic communities in the natural environment. We used benthic chambers to artificially induced anoxia for

different periods of time. After removal of the chambers, we investigated the recolonization patterns of nematode communities. Complementary studies with the same experimental set-up showed a change in the geochemistry of the sediment and the overlying water associated with the development of anoxia (Metzger et al., 2014). Copepod densities and species richness declined (De Troch et al., 2013; Grego et al., 2014) during the experimentally induced anoxic events while foraminifera (protozoan meiofauna) were more resistant (Langlet et al., 2014). Similar experiments, in the same area and using similar experimental equipment also showed in a decrease of macrofaunal density and species richness (Riedel et al., 2012, 2014) after 41 days.

Nematodes were found as the most abundant meiofauna group in the samples (De Troch et al., 2013; Grego et al., 2013) but a detailed analysis of their response is not available yet. Therefore, we investigated the response of the nematode community (in terms of total density and vertical distribution, diversity and community structure) to induced short and long-term anoxia and further evaluate the process of recovery of the nematode assemblage. We tested the hypotheses that, i) the duration of *in situ* anoxia does not affect the nematode community characteristics (density, diversity and vertical distribution, and ii) the recovery of the nematode community is not related to the duration of anoxia.

2. Materials and methods

2.1. Study area

The northern Adriatic Sea (Mediterranean) combines most features that characterise coastal ecosystems sensitive to hypoxia and anoxia (e.g. semi-enclosed basin, shallow

depth, soft bottom, high productivity and stratification, Malej and Malacic, 1995). Moreover, the region shows classical symptoms of long-term anthropogenic eutrophication (Justic, 1987) including repeated animal mortalities (e.g. Stachowitsch, 1984; Hrs-Brenko et al., 1994) and is particularly interesting in featuring both a well-developed infauna and macroepifauna (Zuschin and Stachowitsch, 2009). The experiments were conducted in the Gulf of Trieste, northern Adriatic Sea (45° 32.90´ N, 13° 33.00´ E), about 2 km offshore at a water depth of 24 m in silty sand bottom, near the oceanographic buoy of the Marine Biology Station in Piran, Slovenia (Fig. 1). Normoxic sediment (190 µmol l^{-1}) pore water profiles indicated a low concentration of NH_4^+ (20 - 115 µmol l^{-1}), PO_4^{-3}(2 - 40 µmol l^{-1}) and NO_3^- (5 - 35 µmol l^{-1}) in the sediment (Koron et al., 2015). This station had not experienced oxygen stress before our experiments (Travizi and Vidakovic, 1994; Travizi, 1998, 2000; Riedel et al., 2014).

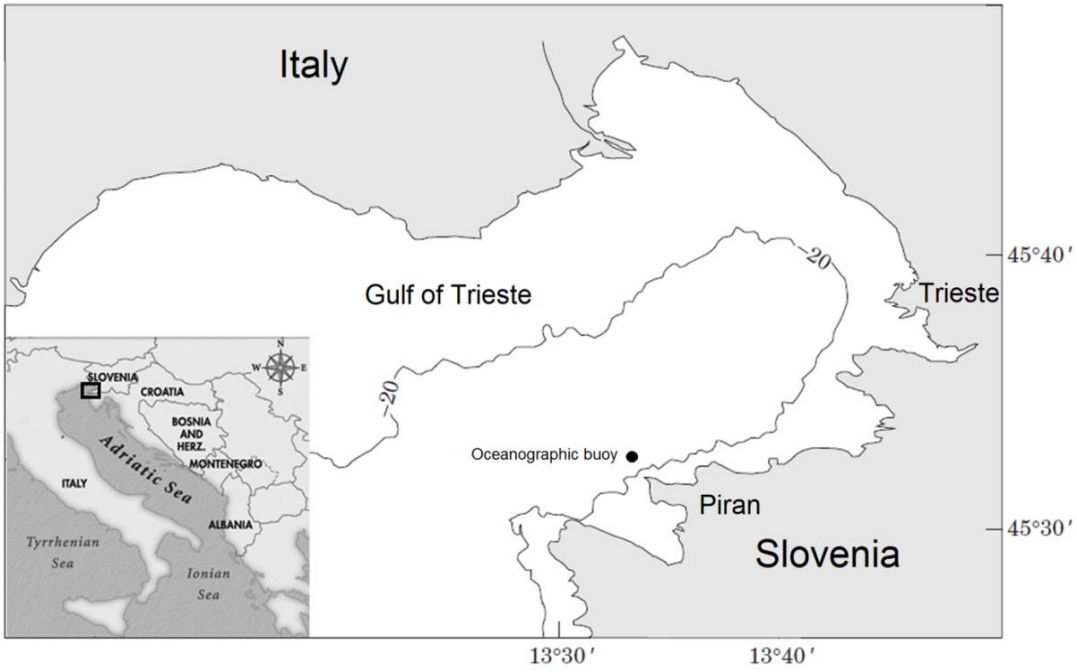


Figure 1. Study site showing the location of the experimental area in silty sand sediment at a depth of 24 m in the Gulf of Trieste (northern Adriatic Sea).

2.2. Experimental set-up and sampling

The experimental set-up has been adapted from an earlier macrofauna study (Riedel et al., 2014). In these experiments, a 50x50x50 cm fully equipped benthic chamber (time-lapse camera, flashes, data logger and sensor array) enabled to experimentally induce hypoxia/anoxia and to document the associated changes in behavioural patterns, sensitivity and tolerance levels of the stressed in- and epifauna (Riedel et al., 2012, 2014, for a detailed description of the method see also Stachowitsch et al., 2007). In the current study except that equipped benthic chamber, two additional chambers (distance between the 3 chambers < 10 m) were used to artificially induce anoxia for 3 different periods of time (Fig. S1). Chambers walls were pushed four centimeters into the sediment and additionally, 20 cm-high inox steel plates were pushed into the sediment parallel to the walls. Note that a specific aspect of the overall project was that metazoan (nematodes and copepods) and protozoan (Foraminifera) meiofauna were studied in the same samples (De Troch et al., 2013; Grego et al., 2014; Langlet et al., 2014).

Three chambers were deployed and sampled respectively on: 2 and 11 August 2010 (ID: Anoxia 9D), on 27 July and 25 August 2010 (ID: Anoxia 1M) and on 24 September 2010 and 5 August 2011 (ID: Anoxia 10M) by scuba divers. Due to the depth of the experiment (24 m), diving constraints (decompression sickness), weather condition and boat availability, deployment times of the chambers did vary by a few days. The first chamber, originally designed to document macroepi- and infauna behaviour was equipped with a detachable instrument lid bearing a digital camera, flashes and sensors (oxygen, H_2S, temperature and pH). In this deployment sensors were positioned 2 cm above the sediment surface (measurements taken in 1 min intervals). The oxygen

concentration started to decrease as soon as the chamber was closed; hypoxia was reached after four days. The real anoxic condition was established after seven days (Metzger et al., 2014). Note that “9 days, 1 month and 10 months” anoxia refer to the duration of the entire treatment from deployment till actual sampling and not to the period of the actual anoxia. As these factor names are used in other papers reporting in the same experiment (De Troch et al., 2013; Grego et al., 2014; Langlet et al., 2013, 2014), we mentioned them here as well in order to facilitate comparison of results. Actual duration of anoxia is mentioned in Table 1. According to the geochemical data, anoxia persisted in both additional chambers as well (Metzger et al., 2014). From every chamber, three replicate cores were obtained immediately after opening. Furthermore, triplicate and duplicate normoxic reference cores were taken on 3 (Normoxia 1) and 25 (Normoxia 2) August 2010 at a distance of 4 to 5 m from the chambers.

Table 1. Deployment and sampling dates as well as real anoxia durations and recovery in the three different benthic chambers.

Samples ID	Deployment day	Sampling day	Real anoxia duration	Recovery duration
Normoxia 1	03. 08. 2010			
Normoxia 2	25. 08. 2010			
Anoxia 9 days	02. 08. 2010	11. 08. 2010	2 days treatment	7 days treatment
Anoxia 1 month	27. 07. 2010	25. 08. 2010	23 days treatment	30 days treatment
Anoxia 10 months	24. 09. 2010	05. 08. 2011	307 days treatment	90 days treatment

After opening, the benthic chambers were removed to allow sediment reoxygenation and re-colonization. The impacted area (where the chambers were placed and anoxic core sampled) was marked with sticks at the corners. Three replicate cores were randomly taken from the intact sediment to investigate the recovery pattern after 7 days

(on 18 August 2010, from Anoxia 2D impacted area), after 30 days (on 23 September 2010, from Anoxia 23D impacted area) and after 90 days of chamber opening (on 03 November 2011, from Anoxia 307D impacted area). All cores (i.d=4.6 cm) were sampled by scuba divers and transported to the laboratory in cooling boxes. Within 4 hours after sampling, all cores were sliced in 0.5 cm intervals for the top 2 cm, and in 1 cm intervals between 2 and 5 cm depth. Samples were stored in a 4% formaldehyde solution.

2.3. Laboratory processing

In the laboratory, each sediment slice was sieved through a 1 mm and a 38 µm sieve. The fraction remaining on the 38 µm sieve was centrifuged three times with Ludox (specific gravity of 1.18) to separate meiofauna organisms from the sediment. We used nematode samples already extracted together with the copepods. Then nematodes separated from copepods and were stained with Rose Bengal and counted (Vanaverbeke et al., 2004). From two replicates, 120 nematodes (or all nematodes if lower number was observed) were hand-picked randomly (Vanaverbeke et al., 2004). Nematodes were transferred to glycerine and mounted on slides for identification to species level according to the pictorial key of Platt and Warwick (1983, 1988), Warwick et al. (1998) and the NeMys online identification system (Vanaverbeke et al., 2015) and library of original species descriptions of the Marine Biology Research Group of Gent University. The identified nematodes were also categorized in to juvenile (J), non-gravid female (F), gravid female (GF), and male (M). Rose Bengal is a stain that adheres to (cytoplasmatic) proteins (Bernhard et al., 2006) and can stain recently dead but not yet

decomposed copepods and nematodes as it can stain proteins in remnants of tissues. This can lead to an overestimation the number of living nematodes (Grego et al., 2013). However, it is shown that Rose Bengal colors living nematodes with a much greater intensity than recently deceased ones (probably duo to more intact proteins), indicating that the species were indeed alive (Danovaro et al., 2010). Therefore, in this study only morphologically intact (not broken and no signs of degradation) and intensely stained specimens were enumerated as alive nematodes (Steyaert et al., 2005; Danovaro et al., 2010). Whenever dead nematodes were observed, they were excluded from further analysis. Finally, based on the morphology of the buccal cavity, the nematodes were allocated to 4 feeding types: (1A) selective deposit feeders, (1B) non-selective deposit feeders, (2A) epistrate feeders, (2B) predators/omnivores (Wieser, 1953).

2.4. Data analysis

For statistical analyses of nematode vertical distribution patterns, community structure and diversity indices, every two 0.5 cm interval slices (from upper 2 cm) were combined into 1.0 cm slices (0-1 and 1-2 cm) yielding comparable sediment sample sizes across the sediment column. Species richness (S), Shannon diversity (H', log e) and evenness (Pielou's J) were calculated for all slices, and for the total sediment column, using PRIMER v6 with PERMANOVA+ add-on software (Anderson et al., 2008). All parametric analyses and graphs were performed and drawn with the freely available R 2.14.2 software (http://www.r-project.org) and results were expressed as mean ± standard error.

2.4.1. The effect of anoxia on marine nematode community

We tested the effect of the anoxic treatment on total nematode density (0-5 cm) by applying a One-way Analysis of Variance (ANOVA). Homogeneity of variances was checked with the Levene's test. When overall significant differences were observed, a Tukey HSD test was used for pairwise comparisons. As assumptions for parametric analyses were not fulfilled, differences in total species richness and Shannon (H', log e) diversity and Pielou evenness (J) among treatments were compared using One-way permutational ANOVA (Permanova) based on a Euclidean distance based similarity matrix. Whenever significant differences between treatments were observed, pairwise tests were performed to investigate differences between pairs of treatments. Due to the restricted number of possible permutations in pairwise tests, p-values were obtained from Monte Carlo (MC) test (Anderson and Robinson, 2003).

Possible differences in the vertical profiles of nematode density, species richness, diversity indices (univariate) and community structure (multivariate) among anoxic treatments were investigated using a fully crossed three factor design using PERMANOVA, following Braeckman et al. (2011b). The design included Treatment (Tr) and slice (Sl) as fixed factors and random factor Replicate (Re) nested in Treatment (Tr). Euclidean Distance and Bray-Curtis based resemblance matrices were used for univariate and multivariate nematode data, respectively. The interaction term Tr×Sl gives information about the difference in depth profiles of nematode densities or community structure among treatments. Whenever significant differences were observed, pairwise tests (p-values were obtained from Monte Carlo test) of Tr within Tr×Sl were performed to investigate in which slice the treatments differed (Anderson

and Robinson, 2003). Homogeneity of multivariate dispersion ('variance') was tested with PERMDISP for any of the significant terms in Permanova analyses. Non significant PERMDISP results indicate a significant PERMANOVA to be a difference due to location. A non-metrical Multidimensional scaling plot (MDS) based on Bray-Curtis similarity visualized the community structure. All multivariate analyses were executed using 4^{th} root transformed data (to reduce the importance of dominant species to the similarity). Furthermore, the similarity percentage analysis procedure (SIMPER) based on 4^{th} root transformed species abundance data of the full sediment column (0-5 cm) was used to identify the nematode species having important contributions to within group similarity. Cut of level for a low contribution was 50% (Clarke and Warwick, 1994).

2.4.2. The recovery of marine nematode community following anoxia

In the present study "7 days", "30 days" and "90 days" recovery refer to the period between removing the chambers until obtaining samples from the first, second and third chamber, respectively. As the starting situation for recovery is different between the different treatments, we limit our comparisons to the patterns observed within a single treatment (Anoxia 2D – Recovery 7D, Anoxia 23D – Recovery 30D and Anoxia 307D – Recovery 90D), and with the reference situation. A three-year study in the Gulf of Trieste did not show an interannual difference in nematode density in summer (Cibic et al., 2009) and we therefore used the nematode community sampled in normoxic conditions (on 3 (Normoxia 1) and 25 (Normoxia 2) August 2010) as reference situations. The nematode community characteristics in both normoxic cores were

analysed. No difference between communities after recovery with the reference samples then reflects full recovery of the community. Univariate (total and vertical density, Species richness, Shannon diversity and evenness) and multivariate (community structure) responses between treatments were investigated using the same designs as explained for testing the effect of anoxia. The SIMPER routine based on 4^{th} root transformed species abundance data was used to investigate which species were responsible for similarity within group among treatments on the full sediment column (0-5 cm). Cut of levels for low contributions were 50% (Clarke and Warwick, 1994).

3. Results

3.1. Effect of anoxia

Total nematode density was statistically different among treatments (one-way ANOVA, $F_{4,8} = 18.10$, $P = 0.000$). Total nematode density was significantly lower after 23 and 307 days anoxia compared to the density in both the Normoxia and Anoxia 2D treatments (Tukey HSD, $p < 0.05$, Fig. 2A). Species richness was considerably affected by the anoxic treatment as well (PERMANOVA, Pseudo-F = 58.52, P = 0.008). In comparison with Normoxia1, the species richness decreased significantly in the Anoxia 23D treatment and it was markedly lower in Anoxia 307D, compared to all other treatments (pairwise test, P (MC) < 0.05, Fig. 2B).

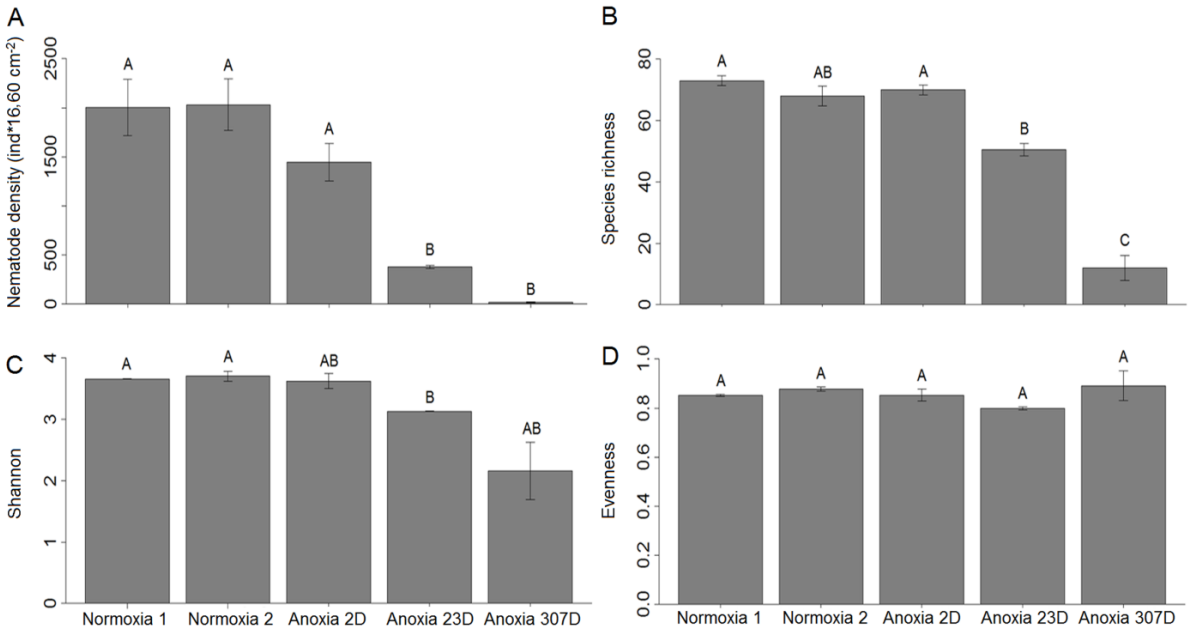


Figure 2.Total nematode density (A) (mean±SE, n=3), species richness (B), Shannon diversity (C) and evenness (D) at all treatments (mean±SE, n=2). Different capital letters above the columns indicate statistically significant results (A>B>C) of pairwise test ($p < 0.05$).

A considerable difference was also observed in Shannon diversity among treatments (PERMANOVA, Pseudo-F = 5.94, P = 0.012). It was significantly lowest in Anoxia 23D though it was comparable with Anoxia 2D and Anoxia 307 (P (MC) < 0.05, Fig. 2C). The evenness index was not significantly affected (PERMANOVA, Pseudo-F = 0.94, P = 0.526) by the different treatments (Table S1 and S2, Fig. 2D).

The vertical distribution of the nematode density was significantly affected by the Treatment×Slice interaction (Fig. 3, Table S3) while PERMDISP test was not significant (PERMDISP, F = 8.27, P (perm) = 0.060). In general, highest densities were observed in the Normoxia and Anoxia 2D cores in the upper 3 cm of the sediment in comparison with anoxia 23 and 307 days. With the exception of the 4-5 cm layer, significant decreases were observed in all other sediment layers in the Anoxia 23D treatments. After 307 days, the density in the deepest layer also decreased. The vertical distribution

patterns of species richness, Shannon diversity and evenness were considerably different among the Treatment×Slice interaction term (Table S3, Fig. 3). Compared to the Normoxia samples, species richness was only significantly affected in all layers in the Anoxia 307D treatment. A decrease in Shannon diversity was only noted in layers deeper than 1 cm, in some treatments. Evenness changed significantly in first layer (0-1cm), the lowest value was observed in the Anoxia 23D treatment (Fig. 3).

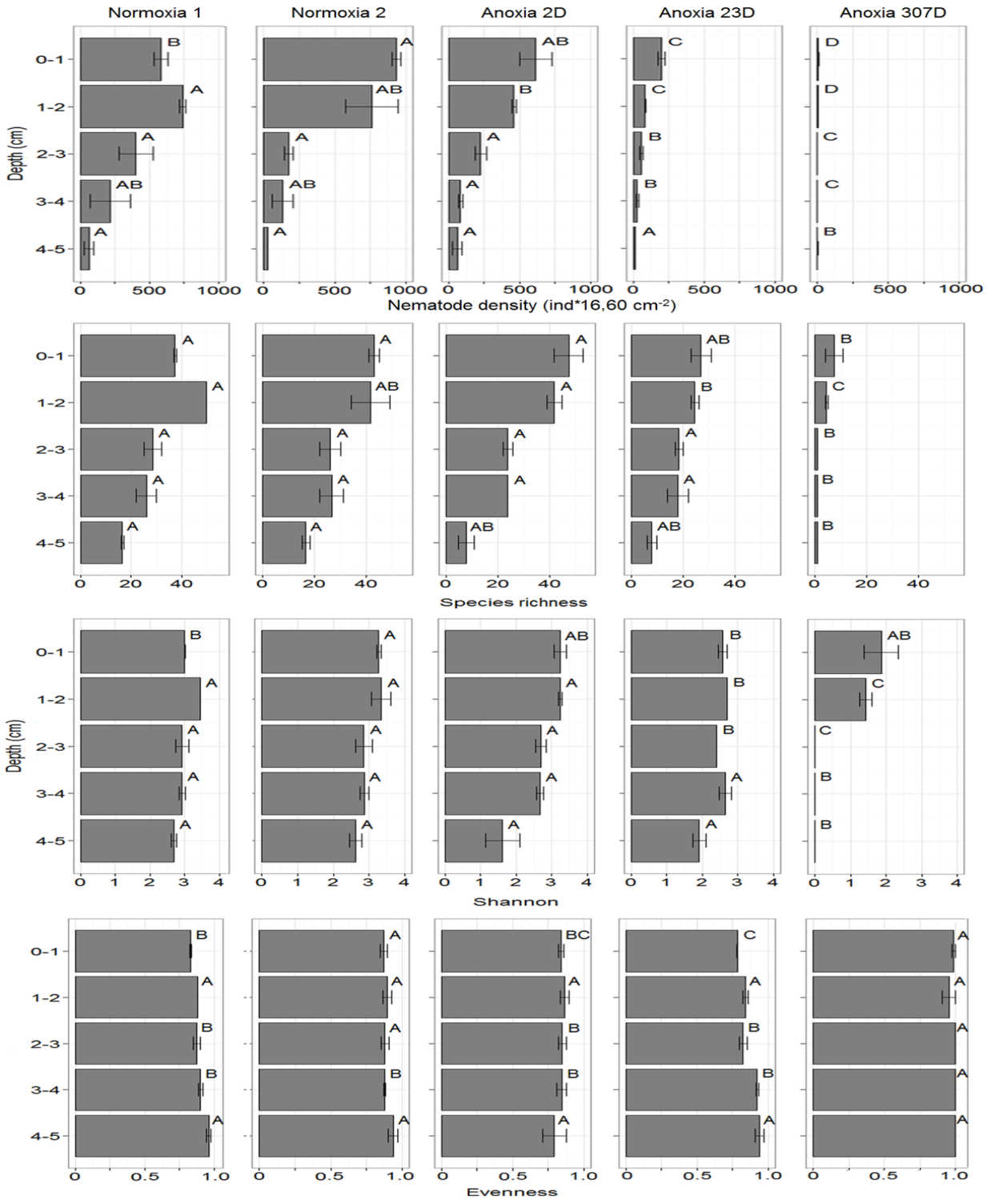


Figure 3. Vertical density profiles (mean±SE, n=3), species richness, Shannon diversity and evenness (mean±SE, n=2) at all treatments. Different capital letters indicate significant differences (A>B>C>D) in the same depth layers ($p < 0.05$).

Nematode community structure was significantly affected by the TreatmentxSlice interaction (Table S3 and Fig. S2). Pairwise tests revealed statistically significant differences between the community from the upper sediment layer at Normoxia 2 and Anoxia 23D and between the community living in the deepest sediment layer from Normoxia 2 and Anoxia 307D ($p < 0.05$).

The results of the SIMPER analysis showed that some species like *Sabatieria celtica*, *Parodontophora* sp1 and *Marylynnia complexus* showed the highest contribution to the low similarity within each of the Normoxia treatments. *Terschellingia longicaudata* was only important in Normoxia 1. As soon as oxygen stress starts to develop, *Theristus longisemicaudatus*, *T. longicaudata* and *Metalinhomoeus effilatus* were important for the similarity in the nematode community. In tha Anoxia 307D treatment, *M. effilatus* explained 79% of the similarity in this treatment (Table 2).

Table 2. Results of the one way SIMPER analysis for the nematode communities and feeding types per treatments in the full sediment column (0-5 cm), FT= feeding type, Cont= contribution.

Normoxia 1			**Normoxia 2**		
Average similarity: 13.38			Average similarity: 13.86		
Species	FT	Cont%	Species	FT	Cont%
Sabatieria celtica	1B	11.89	*Terschellingia longicaudata*	1A	15.76
Parodontophora sp.1	1B	8.42	*Sabatieria celtica*	1B	7.15
Marilynia complexus	2A	7.71	*Sabatieria ornata*	1B	5.18
Metalinhomoeus effilatus	1B	7.12	*Parodontophora* sp.1	1B	4.59
Dorylaimopsis mediterranea	2A	5.93	*Dorylaimopsis mediterranea*	2A	4.26
Theristus longissimecaudatus	1B	5.19	*Richtersia staresensis*	1B	4.20
Actinonema pachydermatum	2A	4.71	*Marilynia complexus*	2A	3.96
			Metalinhomoeus effilatus	1B	3.93
			Spilophorella euxina	2A	3.32

Feeding type contribution (%)			Feeding type contribution (%)		
	1A	0		1A	15.76
	1B	32.62		1B	25.05
	2A	18.35		2A	11.54
	2B	0		2B	0

Anoxia 2D			**Recovery 7D**		
Average similarity: 14.72			Average similarity: 15.40		
Species	FT	Cont%	Species	FT	Cont%
Theristus longissimecaudatus	1B	16.15	*Metalinhomoeus effilatus*	1B	14.05
Terschellingia longicaudata	1A	10.48	*Theristus longissimecaudatus*	1B	7.38
Calomicrolaimus compridus	2A	9.93	*Molgolaimus allgeni*	1A	7.38
Metalinhomoeus effilatus	1B	7.39	*Daptonema fistulatus*	1B	6.77
Spilophorella euxina	2A	4.99	*Sabatieria ornata*	1B	6.76
Sabatieria celtica	1B	4.60	*Dorylaimopsis mediterranea*	2A	5.10
			Marilynia complexus	2A	4.42

Feeding type contribution (%)			Feeding type contribution (%)		
	1A	10.48		1A	7.38
	1B	28.14		1B	34.96
	2A	14.92		2A	9.52
	2B	0		2B	0

Anoxia 23D			**Recovery 30D**		
Average similarity: 16.83			Average similarity: 22.83		
Species	FT	Cont%	Species	FT	Cont%
Terschellingia longicaudata	1A	25.25	*Terschellingia longicaudata*	1A	27.68
Metalinhomoeus effilatus	1B	10.97	*Parodontophora* sp.1	1B	10.20
Theristus longissimecaudatus	1B	10.55	*Richtersia staresensis*	1B	6.79
Linhystera sp.1	1A	7.35	*Spilophorella euxina*	2A	6.09

Feeding type contribution (%)			Feeding type contribution (%)		
	1A	32.6		1A	27.68
	1B	21.52		1B	16.99
	2A	0		2A	6.09
	2B	0		2B	0

Anoxia 307D			**Recovery 90D**		
Average similarity: 12.50			Average similarity: 16.48		
Species	FT	Cont%	Species	FT	Cont%
Metalinhomoeus effilatus	1B	79.09	*Parodontophora* sp.1	1B	17.87
			Theristus longissimecaudatus	1B	11.04
			Terschellingia longicaudata	1A	7.78
			Sabatieria celtica	1B	7.52
			Actinonema pachydermatum	2A	4.85
			Daptonema flagellicauda	1B	4.45
Feeding type contribution (%)			Feeding type contribution (%)		
	1A	0		1A	7.78
	1B	79.09		1B	40.88
	2A	0		2A	4.85
	2B	0		2B	0

Similarity in both the Normoxic and the Anoxia 2D treatments was mainly caused by nematodes belonging to selective deposit feeders (1B) with their contribution about two times higher than the contribution of any of the other groups. At the Anoxia 23D treatment, 2A (epistrate feeders) nematodes were not among the important contributors to the similarity, and an increased contribution of the selective deposit feeders group (1A) was observed (*T. longicaudata*). At Anoxia 307D, non-selective deposit feeder (1B) was the only important feeding type for within group similarity.

In total, 21 species were still present with low densities (mostly juveniles) in the Anoxia 10M treatment. Among them, 12 species inhabited the first centimetre, while two species *M. effilatus* and *Paralinhomoeus caxinus* lived exclusively between 2 and 5 centimeters depth (Table 3).

Table 3. Mean density (M±SE) and vertical distribution of nematode species at 307D Anoxia treatment. In Gender column, the total number (in two replicates) and different stages: juvenile (J), non-gravid female (F), gravid female (GF), and male (M) were reported.

Species	**0-0.5**	**0.5-1**	**1-1.5**	**1.5-2**	**2-3**	**3-4**	**4-5**	**Gender**
Actinonema fidatum	0±0	0.5±0.5	0±0	0±0	0±0	0±0	0±0	1J
Actinonema pachydermatum	0±0	0±0	0±0	1.5±1.5	0±0	0±0	0±0	3J
Antomicron sp.1	0±0	0±0	0.5±0.5	0±0	0±0	0±0	0±0	1J
Camacolaimus tardus	1±1	0.5±0.5	0±0	0±0	0±0	0±0	0±0	1J, 2M
Daptonema lata	0±0	0±0	0±0	1.5±1.5	0±0	0±0	0±0	2J, 1GF
Daptonema sp.1	0±0	0±0	0.5±0.5	0±0	0±0	0±0	0±0	1J
Halaphanolaimus harpaga	0±0	0±0	0.5±0.5	0±0	0±0	0±0	0±0	1M
Leptolaimoides sp.3	0±0	0±0	0.5±0.5	0±0	0±0	0±0	0±0	1M
Marylynnia complexus	0.5±0.5	0±0	0±0	0±0	0±0	0±0	0±0	1J
Metalinhomoeus effilatus	0.5±0.5	0.5±0.5	0±0	0±0	0.5±0.5	0.5±0.5	1.5±0.5	6J, 1M
Nannolaimoides decoratus	0.5±0.5	0±0	0±0	0±0	0±0	0±0	0±0	1M
Odontophora fatisca	0±0	0.5±0.5	0±0	0±0	0±0	0±0	0±0	1GF
Paralinhomoeus caxinus	0±0	0±0	0±0	0±0	0.5±0.5	0.5±0.5	0±0	2J
Promonhystera sp.1	0.5±0.5	0.5±0.5	0±0	0±0	0±0	0±0	0±0	1M, 1GF
Richtersia staresensis	0.5±0.5	0.5±0.5	0±0	0±0	0±0	0±0	0±0	2J
Sabatieria celtica	0.5±0.5	0.5±0.5	0±0	0±0	0±0	0±0	0±0	2J
Setosabatieria hilarula	0.5±0.5	0±0	0±0	0±0	0±0	0±0	0±0	1J
Terschellingia longicaudata	0±0	0±0	0.5±0.5	0±0	0±0	0±0	0±0	2J, 1GF
Theristus sp.1	0±0	0±0	0.5±0.5	0±0	0±0	0±0	0±0	1M
Tricoma sp.1	0.5±0.5	0±0	0±0	0±0	0±0	0±0	0±0	1M
Viscosia elegans	0.5±0.5	0±0	0±0	0±0	0±0	0±0	0±0	1J

3.2. Recovery of marine nematode communities

3.2.1. Recovery after “Anoxia 2D”

When comparing the nematode communities from the Recovery 7D (Anoxia 2D) experiment with the corresponding depths in the Normoxic cores or the Anoxia 2D treatment itself, there were no significant differences in total nematode density, species richness and diversity indices (Table S4, Fig. S3). Likewise, there were no significant differences in the vertical distribution patterns of density, species richness and diversity

indices among interaction (Tr×Sl) terms (Table S6, Fig. S4). There was also no significant difference in nematode community structure (Table S5, Fig. S5).

3.2.2. Recovery after “Anoxia 23D”

A comparison of total nematode density from the Recovery 30D cores with the corresponding values from Anoxia 23D or the Normoxic cores revealed a significant difference (one-way ANOVA, $F_{3,7} = 14.95$, $P = 0.001$). Nevertheless, there were no differences in nematode density of Anoxia 23D treatment and Recovery 30D, but both of them were markedly lower than those in the Normoxic cores (Tukey HSD, $p < 0.05$, Fig. 4A). Overall, there were significant differences in species richness among treatments (PERMANOVA, Pseudo-F = 13.31, P = 0.015). Species richness significantly increased (P (MC) < 0.05) after 30 days recovery, and reached similar levels as in the reference cores (Fig. 4B). There was also a considerable difference in Shannon diversity among treatments (PERMANOVA, Pseudo-F = 27.49, P = 0.004). Shannon diversity increased markedly after 30 days recovery, but it was still significantly lower than in the Normoxic cores (P (MC) < 0.05, Fig. 4C). The evenness index was significantly affected (PERMANOVA, Pseudo-F = 29.57, P = 0.004) by the different treatments as well. Pairwise comparison showed no difference between recovery and anoxia treatments but they were significantly lower than in the Normoxic treatments (Table S6, Fig. 4D).

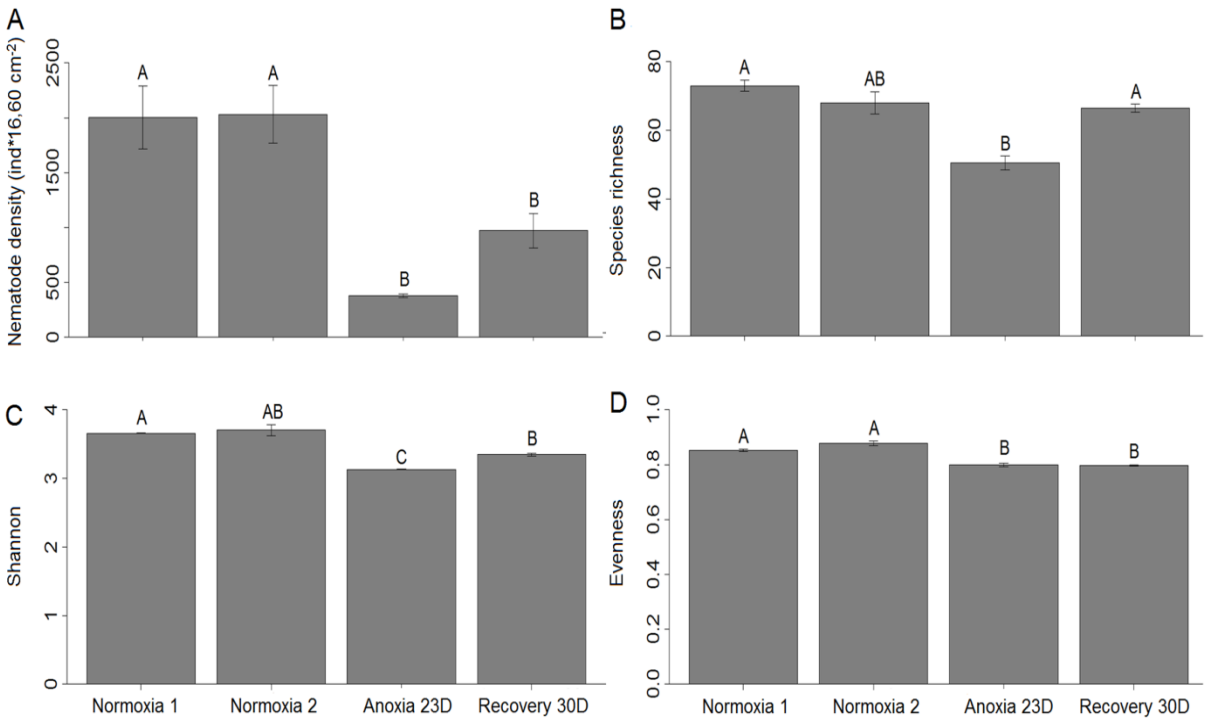


Figure 4. Total nematode density (A) (mean±SE, n=3), species richness (B), Shannon diversity (C) and evenness (D) after 30 days recovery (mean±SE, n=2). Different capital letters above the columns indicate statistically significant results (A>B>C) of pairwise test ($p < 0.05$).

Vertically, nematode density was significantly affected by the Treatment×Slice interaction (Table S7). In addition a significant dispersion effect was detected as well (PERMDISP, F = 7.84, P (perm) = 0.022). In the upper cm, there were no significant differences in the nematode densities between the recovery and the Anoxia 23D treatments, but they were both significantly lower compared to the Normoxia samples. Between 1 and 3 cm sediment depth, nematode densities in the recovering cores where markedly higher compared to the Anoxia 23D treatment, but still lower than in the Normoxic treatment. At the deepest layers, no significant differences were observed (Fig. 5). Analyses of the vertical distribution patterns of species richness and diversity indices (H' and J) did not yield any significant differences in Treatment×Slice interaction term (Table S7).

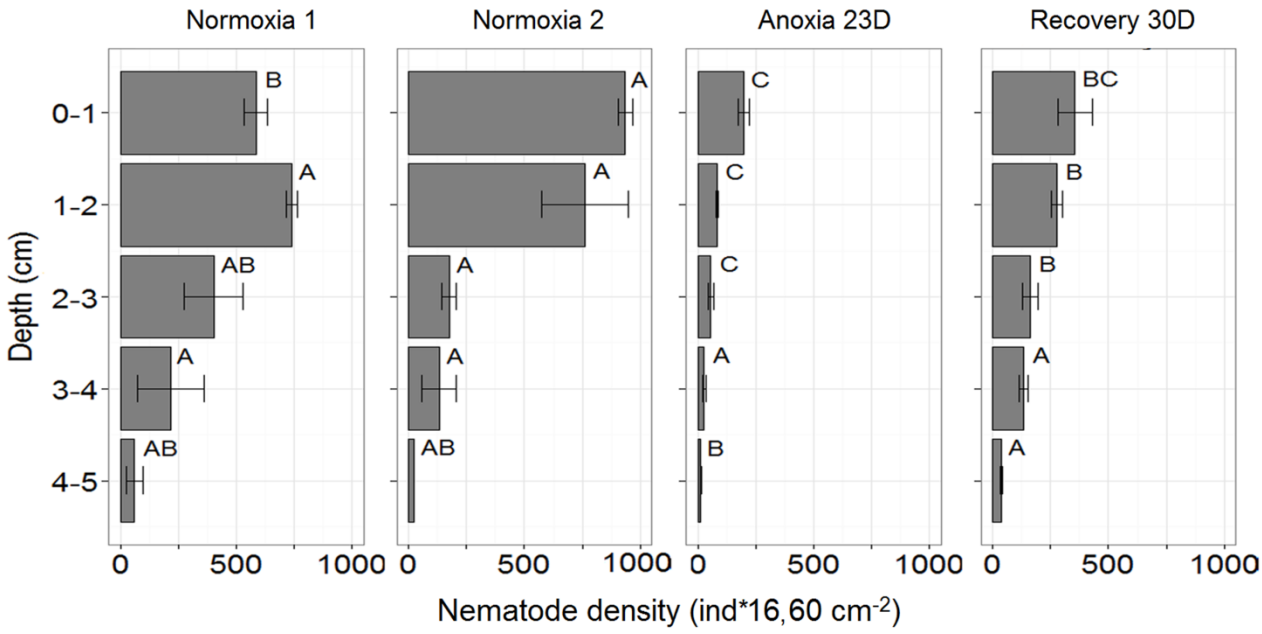


Figure 5. Vertical density profiles (mean±SE, n=3) after 30 days recovery. Different capital letters indicate significant differences (A>B>C) in the same depth layers ($p < 0.05$).

Nematode community structure in the different sediment layers was significantly affected by the Treatment×Slice interaction (Table S7). Pairwise tests only revealed differences between the community from the upper sediment layer at Normoxia 2 and Anoxia 23D (Fig. S6).

The results of the SIMPER analysis showed that *T. longicaudata* (feeding type 1A) was responsible for >25% of similarity in the recovery treatments, as was the case for the Anoxia 23D treatment. However, within the recovery treatment, a 2A-species (*Spilophorella euxina*) was among the important species as well, while this nematode was not a dominant species in the Anoxia 23D treatment (Table 2).

3.2.3. Recovery after “Anoxia 307D”

A period of 90 days recovery (Anoxia 307D) resulted in significant increases in total nematode density and species richness (one-way ANOVA, $F_{3,7}$ = 14.95, P = 0.001 and PERMANOVA Pseudo-F = 53.96, P = 0.001). In addition a non significant dispersion effect was detected for total nematode density (PERMDISP, F = 3.97, P (perm) = 0.467). Total nematode density and species richness values were markedly higher in the Recovery 90D treatments than in the Anoxia 307D cores (Tukey HSD, $p < 0.05$, Fig. 6A) and did not differe from the reference situation. No significant differences were observed for Shannon diversity and evenness (PERMANOVA Pseudo-F = 6.58, P = 0.540 and Pseudo-F = 0.41, P = 0.756, respectively) (Table S8, Fig. 6C and D).

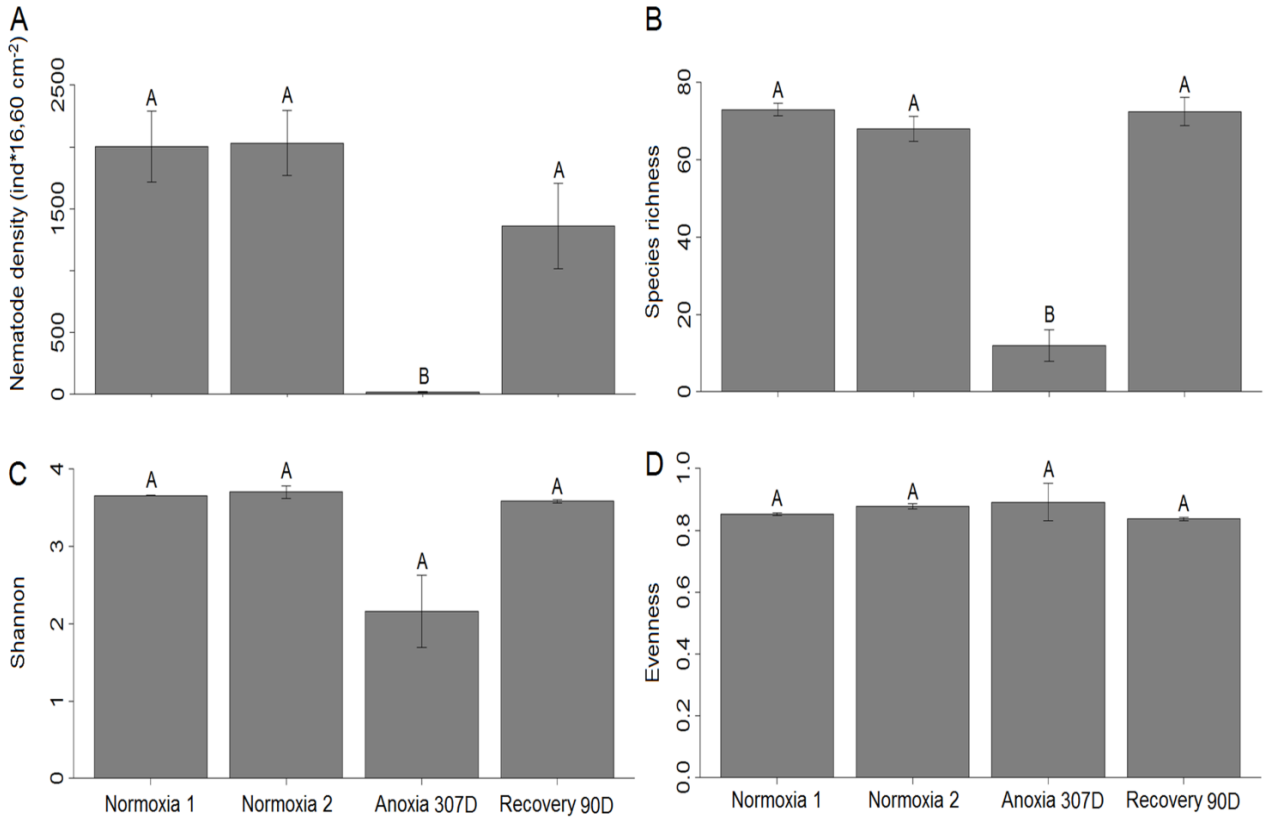


Figure 6. Total nematode density (A) (mean±SE, n=3), species richness (B), Shannon diversity (C) and evenness (D) after 90 days recovery (mean±SE, n=2). Different capital letters above the columns indicate statistically significant results (A>B) of pairwise test ($p < 0.05$).

The vertical distribution of nematode density, species richness and Shannon diversity were all significantly affected by the TreatmentxSlice interaction (Table S9). Nematode densities in the upper 2 cm layers were markedly increased after 90 days of recovery and were not significantly lower than those in the Normoxic treatments. Deeper than 2 cm, there were no significant pairwise differences observed between any of the treatments (Fig. 7). Species richness increased in the upper 3 cm layers compared to the Anoxia 307D treatment and was not significantly different from the values recorded for the Normoxic cores. Shannon diversity showed a rather opposite pattern: here values were considerably higher in all layers below 1 cm depth when compared to the Anoxia 307D treatment, while they were not significantly different from the values recorded for the corresponding layers in the Normoxic samples (Fig. 7). Evenness was not affected by the interaction term (Table S9).

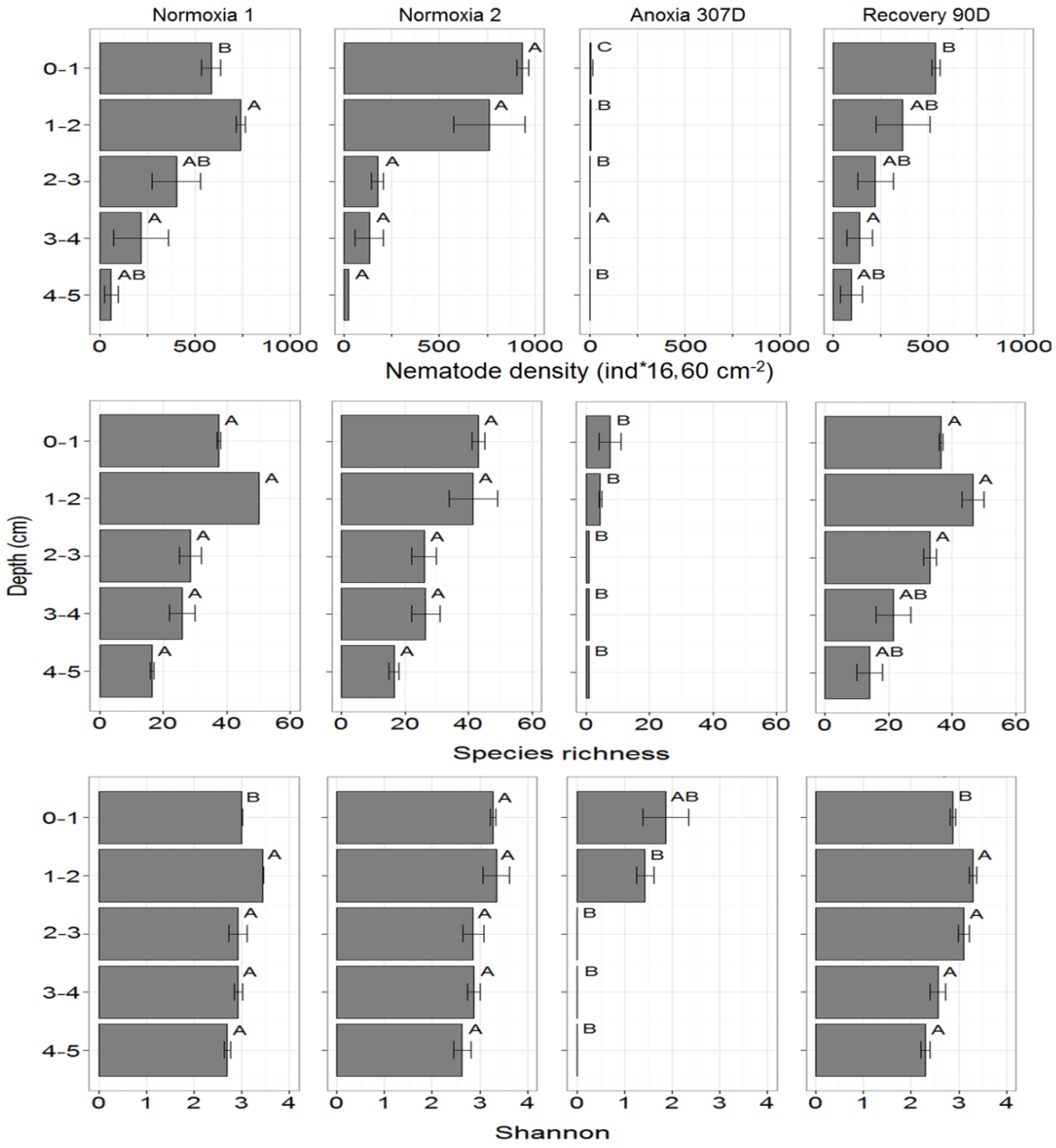


Figure 7. Vertical density profiles (mean±SE, n=3), species richness and Shannon diversity (mean±SE, n=2) after 90 days recovery. Different capital letters indicate significant differences (A>B>C) in the same depth layers ($p < 0.05$).

Nematode community structure was significantly affected by the TreatmentxSlice interaction.There were significant differences between the community from the second (1-2 cm) and the last (4-5 cm) layers at Normoxia 2 and Anoxia 307D (Table S9 and

Fig. S7), which means a full recovery in nematode community after 90 days reoxygenation.

The SIMPER analysis showed that, except *Daptonema flagellicauda,* all species largely contributing to the within group similarity where among the important species for one of the Normoxia treatments. Non-selective deposit feeders (1B) were the most important feeding type, followed by selective deposit feeders (1A) and epistrate feeders (2A) (Table 2).

4. Discussion

Our results showed that short-term anoxia (2 days) did not affect the benthic nematode community characteristics. Nematode density, species richness and diversity decreased after 23 days of anoxia, and decreased further towards 307 days of anoxia. However, live nematodes were still encountered in the latter treatment. *Metalinhomoeus effilatus* was the most dominant nematode species (of the very few specimens) after the long-term exposure (307 days) to anoxia. Twenty three days of anoxia produced substantial effects on sediment geochemistry and thereby potentially driving the observed change in nematodes communities. A complete recovery of nematode communities exposed to 307 days anoxia was observed after 90 days. Therefore, we reject both null hypotheses and discuss our results.

We used underwater benthic Plexiglas chambers to create anoxia in the overlying water and sediment. This was successful, as oxygen concentration in chamber 1 (Sample ID: Anoxia 9 days) started to decrease as soon as the chamber was closed and after four days hypoxia was created. Full anoxia was reached after seven days (Metzger et al.,

2014), hence the organisms in the first chamber were actually exposed to 2 days of anoxic environment. Results of geochemical data confirmed that anoxia was successfully established in both of the additional chambers (Metzger et al., 2014). The treatments caused changes in the geochemistry of the sediment and the overlying waters. There was an upward migration starting from 2 days anoxia in peak concentration of Fe^{2+}, Mn^{2+} and SO_4^{2-} in the sediment and into the overlying waters. Iron peak concentration, for example, migrated upward after 307 days anoxia from 4.5 and 6 centimeter in the Normoxia treatments to 1 centimeter depth in the sediment in Anoxia 307D treatment.

In order to correctly investigate the effect of anoxia on the nematode community characteristics, it is important to unambiguously identify those nematodes that where actually alive at the time of sampling. Therefore, we only took into account individuals that were morphologically intact and intensely stained with Rose Bengal, following Steyaert et al. (2005) and Danovaro et al. (2010). Weakly stained or broken nematodes and those showing signs of degradation were all excluded from analyses. While the use of CellTracker Green Labelling to distinguish dead from life nematodes (and copepods) was supported by Grego et al. (2013), logistical/technical constraints prevented us to explore this method for the current paper. Given the clear patterns observed (sharp decrease in nematode density, strong changes in nematode diversity patterns) and the careful inspection of all nematodes in the samples, we are confident that the patterns reported here reflect the actual response of nematode communities to different periods of exposure to anoxia.

As Foraminifera (Langlet et al., 2013) and harpacticoid copepods (Grego et al., 2014) were investigated in the same cores, we are able to describe how different meiofaunal taxa are affected by different durations of anoxia. Copepods were the most sensitive group, as about 70% of the community died in the Anoxia 2D treatment, and after 23 days less than 10% survived. Species richness decreased drastically as well: only 50% of the species were still present in the Anoxia 2D treatment and this dropped further to 20% after 23 days (Grego et al., 2014). The nematode community did not show such strong response in the Anoxia 2D treatment, but their density and species richness decreased at longer exposure to anoxia. In the Anoxia 23D treatment, about 25% of the nematodes survived, and 50% of the species were still present. Later on (Anoxia 307D), density decreased to 1% of the original values, and 20% of the species remained present. Finally, Foraminifera were the most resistant meiofauna group as about 67% of the community survived in the Anoxia 23D treatment. Species richness decreased slightly so that 89% of the total species were still present in the Anoxia 23D treatment. At Anoxia 307D treatment their density and species richness decreased to 43 and 17% of the original values, respectively. It is necessary to note that certain nematode and Foraminifera (protozoan meiofauna) species survived at the 307 days anoxia.

The different response of these three meiofaunal taxa can be explained by phylogenetic constraints and meiofauna lifestyles (Wetzel et al., 2001). In general copepods have a short and cylindrical body which results in a low length/width ratio. This low ratio decreases oxygen uptake by diffusion (Wetzel et al., 2001; Giere, 2009). Besides, a stop in copepod feeding activity (low energy yield) during anoxia (De Troch et al., 2013) as well as their high respiration rate makes them less resistant (Wetzel et al., 2001) to

oxygen stress. In contrast, a higher length/width ratio (increase oxygen uptake in suboxic condition) as well as low respiration rates of nematodes (Braeckman et al., 2013) together with different kind of adaptations (see below: The responses of nematode community to anoxia) increases their ability to resist anoxia (Wetzel et al., 2001). The ability to survive several weeks of hypoxic and anoxic conditions is also quite widespread among foraminiferans, with different mechanisms involved. The ability to decrease their respiration rates in suboxic condition (Braeckman et al., 2013), for example, along with their ability to switch to an anaerobic metabolism (Moodley and Hess, 1992) makes them more tolerant to anoxia. Moreover, for some species an adaptation to escape temporary environmental change is the upward migration in the sediment, tracking the oxygen and/or sulphide gradient (Duijnstee et al., 2003). Finally, there is also evidence that different life strategies of benthic foraminifera to better survive hypoxic conditions coincide with a morphological change. Numerous studies have reported deviations in the overall test/chamber morphology, i.e. size, shape, porosity and/or pore distribution (Bernhard, 1986; Rathburn and Corlis, 1994) in relation to low oxygen concentration. Also the pore density/number is suggested to be influenced by the bottom-/pore-water oxygen content (Glock et al., 2011). However, these changes make them more vulnerable to dissolution in low pH conditions (Zeppilli et al., 2015). It is shown that low dissolved oxygen and low pH conditions co-occur in many coastal and open ocean environments (e.g. Melzner et al., 2013; Mora et al., 2013; Gobler and Baumann, 2016). Therefore, adaptations to low oxygen conditions might lead to increased vulnerability in future conditions when ocean acidification would result in lower pH values.

4.1. The responses of nematode community to anoxia

Although most marine nematodes prefer to live in oxygenated sediment (Wetzel at al., 2001; Steyaert et al., 2005), some nematode species spend extended periods in hypoxic and anoxic conditions like in the Black Sea (Muresan, 2012; Muresan and Gomoiu, 2012; Sergeeva and Zaika, 2013) or in anoxic deep sediment layers (Moodley et al., 1997; Soetaert et al., 2002; Braeckman et al., 2011a, b; Taheri et al., 2014). In the natural environment, responses of marine nematode communities to oxygen stress are species-specific and related to the duration of oxygen stress, and morphological, behavioural and physiological adaptations (Modig and Olafsson, 1998; Travizi, 2000; Steyaert et al., 2007).

In our first chamber (Sample ID: Anoxia 9 days), a gradual reduction in oxygen concentration from normoxia to hypoxia and further to anoxia was observed during the first 7 days of the experiment (Metzger et al., 2014). In this treatment, we did not observe any nematode migration to more surficial sediment layers, as observed before in experiments imposing short-term oxygen stress (Taheri et al., 2014). Most of marine nematodes are elongated cylindrical worms around 1-2 mm long (Warwick et al., 1998) but a variation in nematode body size and shape in relation to sediment oxygen concentration has been reported (Soetaert et al., 2002). It seems that long and slender nematodes are more adapted to live in less oxygenated environment (Soetaert et al., 2002; Braeckman et al., 2013). Their higher L/W ratio results in higher oxygen absorption efficiency per unit of volume, while longer nematodes have more chance to bridge oxic spots with their body (Soetaert et al., 2002). The respiration rates of the nematodes are directly controlled by oxygen availability in the environment (Braeckman

et al., 2013). Decreasing feeding activity under anoxia (Steyaert et al., 2007) together with lower respiration (metabolism) rates has been reported as adittional adaptation mechanism to oxygen stress in marine nematodes (Ott and Schiemer, 1973; Braeckman et al., 2013). All mentioned adaptation mechanisms and the gradual decrease of oxygen availability can explain the lack of negative response of nematodes to short-term anoxia. Similarly, other studies showed a survival of marine nematodes to short-term hypoxia and anoxia (Guerriniet al., 1998; Steyaert et al., 2005; De Troch et al., 2013; Taheri et al., 2014).

As marine nematodes have aerobic respiration, they cannot survive in a long-term anoxia exposure (Moodley et al., 1997; Wetzel et al., 2001). Apart from some of the already mentioned physiological and morphological adaptations, migration from anoxic sediment to the water column is reported by Wetzel et al. (2001). However, in our experiment, the closed chamber did not allow nematodes to escape from the anoxic area. The first strong decrease in nematode density in the Anoxia 23D treatment coincides with the appearance of large concentrations of H_2S in the overlying water within the closed chamber, probably caused by mineralization of decaying macrofauna at the sediment-water interface (Metzger et al., 2014). However, H_2S concentrations in the sediment at that moment, and at the end of the Anoxia 10M incubation itself were not markedly increased (Metzger et al., 2014), ruling out a possible toxic effect of H_2S (Moodley et al., 1997; Steyaert et al., 2007) on the nematode community in our experiment.

Apart from anoxia, availability and quality of food resurcess should be taken into account. Inducing an anoxic condition through the isolation from surrounding water (no

water exchange) not only directly affected the nematode community but also changed the availability and quality of food (Middelburg and Levin, 2009; Metzger et al., 2014). The interplay of oxygen and food availability could also affect the responses of the nematode community to anoxia (Levin et al., 2009). Although certain diatoms are able to survive (but do not grow) in dark and anoxic sediments (Kamp et al., 2011), it seems that diatom abundance in the Anoxia 23D chamber decreased, due reduced light at 24 m depth in combination with settling sediment and large animals (e.g. brittle stars) on the outer surface of the chambers (Grego et al., 2014; Metzger et al., 2014) and the presence of H_2S (Admiraal and Peletier, 1979; Metzger et al., 2014). Diatoms are an important food source for epistrate feeder nematodes (Moens et al., 2014). Therefore, the low proportion of epistrate feeders in dominant nematode groups at this treatment (Anoxia 23D) could be explained by the lower availability of diatoms as a food source (Austen and Warwick, 1995) in combination with oxygen stress. On the other hand, at this treatment, three deposit feeder species (1A+1B), *Theristus longissimecaudatus, T. longicaudata* and *Metalinhomoeus effilatus* were dominant in the nematode community. The last two species are known to survive long anoxic events (Van Gaever et al., 2009; Vanreusel et al., 2010; Muresan and Gomoiu, 2012). The high level of organic matter input due to decay of infaunal macrofaunal organisms in Anoxia 23D treatment (Metzger et al., 2014) probably increased the abundance of bacteria (Gallizia et al., 2005; de Moraes et al., 2014). The selective deposit feeders (1A) are the main consumers of bacteria in the nematode community (Moens and Vincx, 1997; Neira et al., 2013). Therefore, the increasing relative contribution of this group (because the 2A nematodes die) in the Anoxia 23D treatment could be related to increasing bacterial density inside

the chamber which corroborates the findings of Austen and Warwick (1995). Finally, some non-selective deposit feeders are able to consume bacteria (Ingels et al., 2011) although their aboundance in the Anoxia 23D treatment decreased a little, it seems the available food was sufficient for their survival as well.

The ability to switch between aerobic and anaerobic metabolism has been reported for a few nematode species (Barbercheck and Duncan, 2004; Tahseen, 2012) as an explanation to survive long-term anoxic events. However, the anaerobic metabolism in microorganisms yields only about 12-13 percent of the total achievable energy after complete oxidation of food in the aerobic metabolism (Megonigal et al., 2004). A reduction in feeding activity (Steyaert et al., 2007), in combination with a low energy yield from the available food can lead to unsuccessful reproduction and decreased survival of juvenile nematodes (Austen and Wibdom, 1991). Even for some species like *Theristus anoxybioticus* which is known as a species tolerant to anoxia, oxygen is an essential requirement for reproduction (Jensen, 1995). A longer nematode generation time (four to eight times longer), is reported under anoxic circumstances as well (Jensen, 1995). Hence, in our experiment, the drastic decline in nematode density and species richness from Anoxia 23D to Anoxia 307D treatment could be related to the much lower energy obtainable by surviving nematodes (after Anoxia 23D), a decrease in reproductive success, lower survival of juveniles and longer generation time during 307 days of oxygen stress (Wetzel et al., 2001; Gambi et al., 2009; Van Colen et al., 2009).

Exposure history plays an important role in resistance of marine nematodes to anoxia (Modig and Olafsson, 1998; Wetzel et al., 2001). An area like the gulf of Trieste, where

periodical hypoxia/anoxia during the summer has been reported (Malej and Malacic, 1995; Travizi, 1998, 2000; Giani et al., 2012) has a potential for developing a nematode community with species tolerant to oxygen stress. Therefore, the presence of some resistant species was expected. From the nematodes surviving in the Anoxia 307D treatment, *Camacolaimus* and *Daptonema* (Modig and Olafsson, 1998; Muthumbi et al., 2004; Guilini et al., 2012), *Metalinhomoeus* (Muresan and Gomoiu, 2012), *Odontophora* (Wieserand Kanwisher, 1961; Van Colen et al., 2009), *Richtersia* (Muresan and Gomoiu, 2012), *Sabatieria* (Wieser and Kanwisher, 1961; Modig and Olafsson, 1998; Gambi et al., 2009; Muresan and Gomoiu, 2012), *Terschellingia* (Van Gaever et al., 2009; Vanreusel et al., 2010; Muresan and Gomoiu, 2012) and *Viscosia* (Muresan and Gomoiu, 2012) have been reported previously as being tolerant species to more than two months of oxygen stress in different marine ecosystems from the coastal water till the deep sea. Free-living marine nematodes are often used as biological indicators in marine ecosystem monitoring (Bongers and Ferris, 1999; Boyd et al., 2000; Moreno et al., 2011; Semprucci et al., 2013). Our results showed that resident species at Anoxia 307D could be proposed as indicator of mid- to long-term anoxia. Among surviving species, the non-selective deposit feeding nematode (1B, *M. effilatus*) was dominant in the community. Bacteria, diatoms and even dissolved organic carbon contribute to the diet of this group (Moens and Vincx, 1997; Moens et al., 2014). Being able to exploit a wide range of food sources helps this species surviving in prolonged anoxic conditions.

4. 2. The recovery of marine nematode community

Recovery after anoxia started after removal of the chambers from the sediment, but there is often a lag between the increase in oxygen concentration in the overlaying water and a full recovery of sediment biogeochemistry, especially in relation to H_2S (Wetzel et al., 2002). Although during the recovery process biochemical conditions were not measured in this study, we know that the reoxygenation of previously anoxic sediments can increase the organic matter (i.e. decomposition of macrobethic fauna) mineralization (Bastviken et al., 2004) and, as a consequence, the habitat (sediment) quality will improve. Recovery of nematode community can also be strongly related to the recovery of the local macrobentic community (Van Colen et al., 2009). Owing to macrobenthic bioturbating and bio-irrigating, not only benthic mineralization will enhance (Braeckman et al., 2010) but also food and oxygen can be transported from the surface sediment to deeper layers creating a suitable habitat for nematodes (Braeckman et al., 2011b). An increase in bioturbation activity as a result of recolonization of macrobenthic fauna has indeed observed after anoxia (Karlson et al., 2007).

Our results did not show a negative effect of short-term anoxia (first treatment) on the nematode community, hence no recovery trends were expected here. While species richness markedly improved after 30 days of recovery from the 23D Anoxia treatment, the total density did not fully recover when compared to the Normoxia treatments. A slow nematode recovery process especially after one month anoxia was also reported in the southern Baltic Sea (Wetzel et al., 2002). Nematode recovery mainly occurs through migration rather than by reproduction of individuals thet have survived

(Guerriniet al., 1998; Wetzel et al., 2001). Although some species are able to actively move (lateral migration) in the sediment (Wetzel et al., 2001; Schratzberger et al., 2004; Guilini et al., 2011) with relatively high (2-3 mm s^{-1}) speed (Cullen, 1973), the absence of planktonic larvae in free-living marine nematodes (Platt and Warwick, 1988) is expected to hamper efficient colonization from undisturbed sediments to impacted areas. The continuous reproduction activity of marine nematodes, their low fecundity and their life cycle between 20–30 days (Platt and Warwick, 1988) can explain why notwithstanding the recovery of species richness, the density did not increase. Nevertheless, after 30 days recovery epistrate feeders' reappeared among the dominant nematodes which could be related to increasing numbers of diatoms in the sediment due to their settlement from the water column or arriving by currents after removing the chamber. Although the nematode density did not fully recover, all the dominant nematodes in Recovery 30D treatment were observed in Normoxic sediments as well, indicating that recovery has started from surrounding areas.

With regard to the Recovery 90D treatment (after a 307D anoxic treatment), the nematode total and vertical density, species richness and diversity in the entire sediment column (0 to 5 cm depth) and the upper two cm indicated a full recovery (in comparison with Normoxia treatments) and all three feeding types reappeared in the nematode community. It seems that reoxygenation of sediment (diffusion or advective processes) and macrobenthic activity (bioturbation and bioirrigation) established renewed suitable conditions for inhabiting nematodes (Van Colen et al., 2009). Furthermore, short life cycles (20–30 days) in most marine nematodes with a continuous reproduction might have supported this process (Platt and Warwick, 1988).

The dominant nematode species were almost the same as in the Normoxic treatments, pointing to a full recovery to pre-anoxic conditions.

5. Conclusion

This experimental design allowed us to study the response of the nematode community to different durations of oxygen depletion in the natural environment. Our results showed a species-specific response of marine nematode community to anoxia. While short-term anoxia did not affect the nematode community, a decrease in nematodes density and species richness as well as changes in the feeding type distribution were observed at longer-term anoxia treatments (23 and 307 days). The nematode community at the recovery phase was lightly influenced by the community from the surrounding area as well as abiotic and biotic factors. Considering the dominance of marine nematodes in meiofauna, surviving nematode species at Anoxia 307D treatment (like *M. effilatus* and *P. caxinus*) could be used as indicators of oxygen stress as well.

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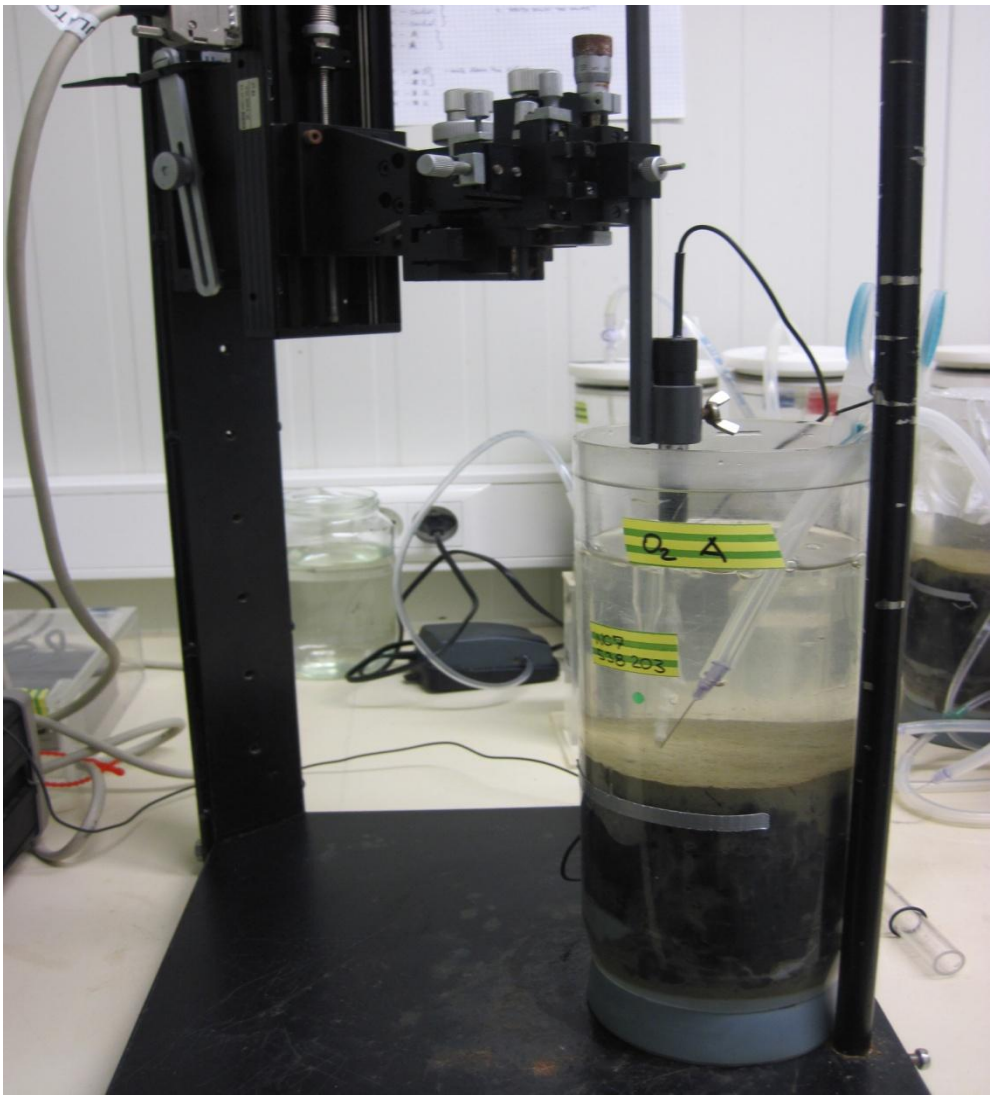
Chapter 4

Effect of short-term hypoxia on the feeding activity of abundant nematode genera from an intertidal mudflat



In prepreation as “Mehrshad Taheri, Marina Giunio, Marleen De Troch, Magda Vincx, and Jan Vanaverbeke.

Abstract

The effect of short-term hypoxia (6 days) on the feeding activity of abundant nematode genera was investigated by means of a tracer experiment. Nematodes were sampled from the Paulina intertidal flat in the Westerschelde estuary (south-west Netherlands) and incubated with ^{13}C pre-labelled diatoms at the sediment-water interface in Oxic and Hypoxic treatments. In general, specific uptake and uptake of carbon per unit of nematode carbon were low in all studied genera which indicated that the added diatoms represented a limited food source for the investigated nematode genera. Results from such a low uptake are difficult to interpret, however based on our results; there was no significant decrease in feeding activity of all dominant nematodes in the Hypoxic treatments. The low carbon uptake might be related to low access of nematodes and their low feeding preference to the added diatoms in the experimental cores.

Keywords hypoxia, intertidal nematodes, feeding activity, stable isotope, food uptake, Westerschelde estuary.

1. Introduction

Hypoxia (oxygen concentration < 63 µmol l^{-1} or < 2 mg l^{-1}) is considered to be a serious problem in coastal waters (Middelburg and Levin, 2009; Vaquer-Sunyer and Duarte, 2010) and is estimated to increase worldwide due to coastal eutrophication, global warming and their combined effects (Tilman et al., 2001; Keeling et al., 2010; Rabalais et al., 2010; Moffitt et al., 2015). Although hypoxia is occurring in the water column, bottom water oxygen depletion affects both biogeochemical processes in the sediment and the benthic faunal life (Middelburg and Levin, 2009; Vaquer-Sunyer and Duarte, 2010; Riedel et al., 2014).

The response of free living marine nematodes (the dominant mieofauna group in most marine ecosystems, Giere, 2009) to overlying water hypoxia is species-specific and related to the duration of hypoxia and a combination of behavioral and physiological adaptations of the nematode species to live in oxygen-stressed environments (Modig and Olafsson, 1998; Travizi, 2000; Steyaert et al., 2007). Most free-living marine nematodes prefer to live in oxygenated sediment (Wetzel at al., 2001; Steyaert et al., 2005) however, some species has been reported in the anoxic deep sediment layers (Moodley et al., 1997; Soetaert et al., 2002; Braeckman et al., 2011b; Taheri et al., 2014) or survived short-term experimental hypoxic and anoxic conditions (Guerrini et al., 1998; Steyaert et al., 2005; Taheri et al., 2014; Taheri et al., 2015). When nematode communities were subjected to longer periods of the hypoxia or anoxia, a change in communities together with a decrease in both density and diversity were observed (Moodley et al., 1997; Van Colen et al., 2009; Taheri et al., 2015).

Oxygen is an essential element for aerobic respiration in order to generate energy. Nematode oxygen uptake is by diffusion and related to the oxygen availability in the environment, which affects nematode respiration rates (Braeckman et al., 2013). Respiration rates (metabolism) of several nematode species exposed to the hypoxia were significantly lower than in nematodes exposed to normoxia (Ott and Schiemer, 1973; Braeckman et al., 2013). As some part of the obtained oxygen is used for complete oxidation of food by the aerobic metabolism, a decreased respiration rate can be related to decreased food uptake. On the other hand, a decreased respiration rate can be caused by a reduced activity or a switch to a partly anaerobic metabolism (Wieser and Kanwishe, 1961; Schiemer and Duncan, 1974).

Traditionally, the feeding types of nematodes have been defined based on mouth morphology to four different group: (1A) selective deposit feeders; (1B) non-selective deposit feeders; (2A) epistrate (diatom) feeders; (2B) predators/omnivores (Wieser, 1953), but a modified feeding type classification was proposed based on observations on nematode feeding behavior (e.g. Moens and Vincx, 1997; Moens et al., 2014). We used the latter classification in this study. We focus on the feeding activity of intertidal nematodes in sediments underlying hypoxic water. We tested the hypothesis that short-term hypoxia (6 days) does not affect the feeding activity of nematode communities; hence it has no direct effect on the energy supply of the nematodes.

2. Materials and methods

2.1. Study area

Samples were collected from an intertidal mudflat (Paulina) in the Westerschelde estuary (south-west of The Netherlands; 51° 21’ 24”N, 3° 42’ 51” E). Long-term trends (1965–2002) in dissolved inorganic nutrients in the tidal part of the Scheldt estuary showed a fluctuating pattern. Annually averaged concentrations of dissolved inorganic nutrients (Si, N and P) significantly declined after mid-1970s. In the early 1970s, high loadings of ammonium and organic matter caused oxygen depletion (Heip, 1988; Baeyens et al., 1998; Soetaert et al., 2006) but with a decrease of the ammonium input in the 1990s, an increase in oxygenation was visible from 1995 onwards (Soetaert et al., 2006). The Paulina intertidal mudflat is located in the polyhaline part (average salinity between 24 and 33) of the Westerschelde estuary (Moens et al., 2002). The mudflat has a gentle slope and a mean tidal range of 3.9 m, with a semidiurnal regime (Ysebaert, 2000). In this area, different sediment types, hydrodynamics, tidal currents and shore vegetation create a wide variety of benthic habitats (Van Colen et al., 2010a). In bare intertidal sediments, nematodes are the most important meiofauna group and their highest density is reported in the upper 2 centimeter layers (Soetaert et al., 1994; Steyaert et al., 2003; Van Colen et al., 2009).

2.2. Sampling and experimental design

In the sampling station sediment median grain size and mud fraction < 63 µm (silt + clay) were 47 µm and 64.42 ± 2.26 % in the 0-1 and 41 µm and 71.61 ± 1.39 % in the 1-2 cm, respectively. Sediment was sampled by means of eight Plexiglas cores (i.d. 10

cm) in the high intertidal zone of the Paulina mudflat in September 2013 (Fig. 1). The bottom of the cores were sealed with rubber stoppers before transfer to the lab (less than two hours), maintained in a temperature controlled room (15 °C) and topped with seawater (salinity 24) from the sampling site. Cores were randomly allocated to the Control treatment (2 cores for $\delta^{13}C$ natural values), the Oxic treatment (3 cores) and the Hypoxic treatment (3 cores) and connected to an air pump overnight. The following morning, the oxygen concentration in the overlying water, and vertical sediment oxygen profiles (three replicates) were measured in the cores from the Oxic and Hypoxic treatments using Unisense oxygen micro sensors (type ox25) in vertical increments of 250 µm.

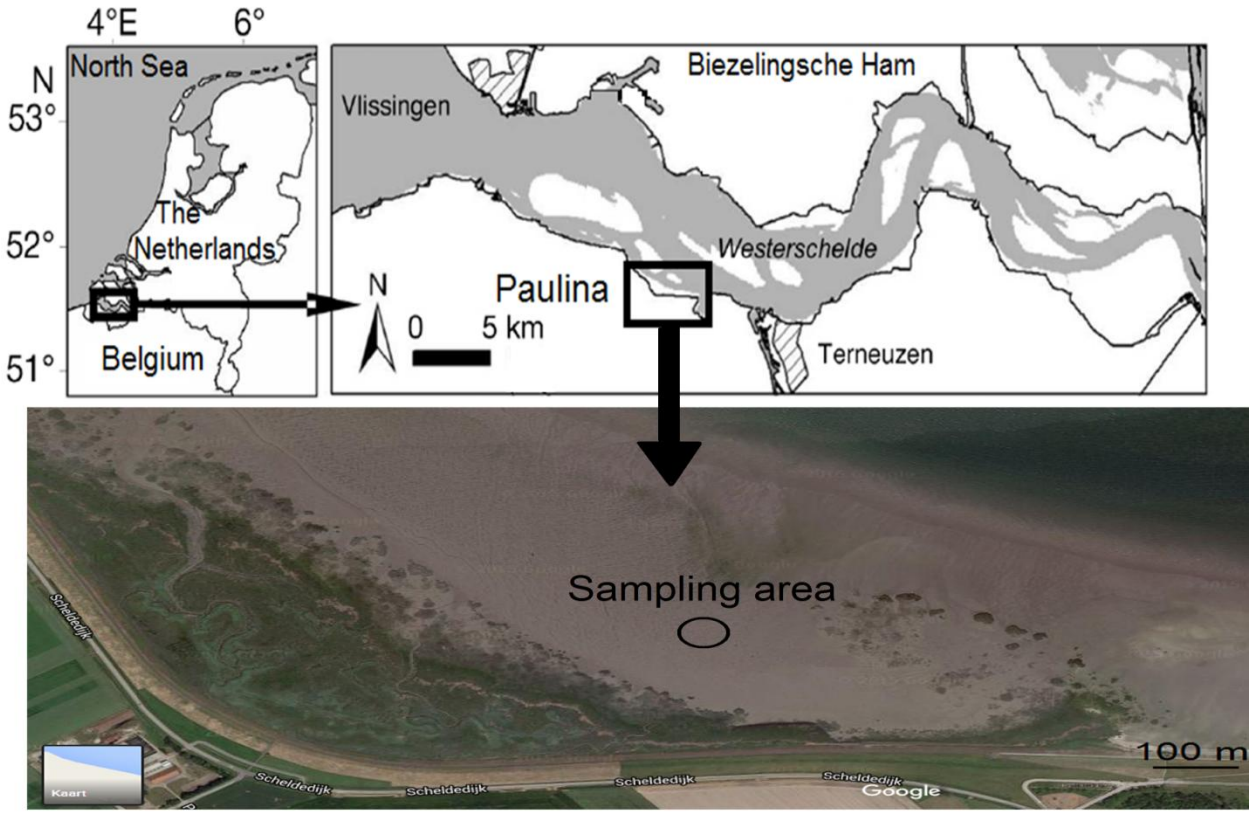


Figure 1. Location of the sampling site, the Paulina intertidal mudflat, in the Westerschelde estuary, south-west of The Netherlands.

To evaluate the feeding activity of nematodes, ^{13}C pre-labelled benthic pennate diatoms *Cylindrotheca fusiformis* (25-30 µm in length, accession number: DCG 0423, in the diatom culture collection of the Laboratory of Protistology and Aquatic Ecology, Ghent University, Belgium) were added on top of the sediment in the Oxic and Hypoxic treatments. The diatoms were labelled with the stable isotope ^{13}C by adding 5mL of a $NaH^{13}CO_3$ stock solution (^{13}C, 99%, Cambridge Isotope Laboratories, 336 mg per 100 ml milliQ H_2O) per 100 mL of the culture medium (Guillard, 1975). The diatom culture was grown in a climate room at 15±1 ˚C with a 12:12 h light–dark regime. At the start of the experiment (day 1) 7.6×10^7 fresh diatom cells (corresponding to 968153 cells and 0.064 mg carbon cm^{-2}) were pipetted homogeneously on the sediment surface of every Oxic and Hypoxic core. Added algal carbon represented 0.65% of the total organic carbon in the first cm of the sediment (rough estimate based on Moens et al., 2002). The labelling technique resulted in 13.74 % ^{13}C in the ^{13}C-enriched cultures. After addition of the labelled diatoms, each core was sealed by a lid (to avoid outgassing and evaporation) with two valves, one as an air inlet and the other as a safety valve. The overlying water was bubbled with nitrogen (97%; Air Liquide Company, Belgium) through aquarium airstones in the Hypoxic treatment and with ambient air in both the Oxic and Control treatments. All airstones were placed 7 cm above the sediment surface in the water column to prevent sediment disturbance and resuspension of diatoms, and to create a very gentle water circulation in the cores (Fig. 2). Then the cores were stored in the dark at a temperature of 15 °C.

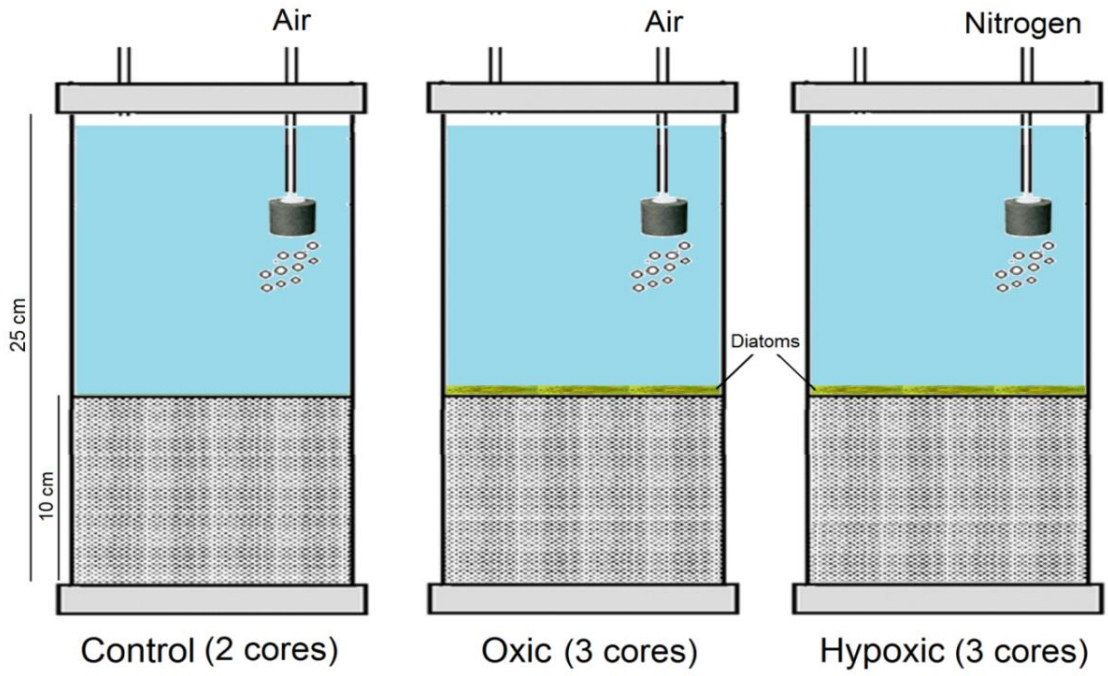


Figure 2. Schematic drawing of the experimental set up. Overlying water was continuously bubbled with air (Control and Oxic treatments) or a nitrogen gas (Hypoxic treatment).

The dissolved oxygen concentrations in the overlying water of two Oxic and two Hypoxic cores were monitored with Oxygen Spot Sensors (OXSP5, Pyroscience) glued to the inner wall (4-5 cm above the sediment surface) of each core before the start of the experiment. The sensors were operated with an optical oxygen meter (FireStingO2, Pyroscience), connected with a lens spot adapter (SPADLNS) and Spot Fiber (SPFIB). At the end of the experiment (after 6 days), the cores were opened and sediment oxygen profiles were measured in three replicates as described above. Then, the overlying water was gently siphoned off, taking care to minimize sediment disturbance, and the upper two centimeters of sediment were sliced in 1 cm intervals (0-1 and 1-2). In the field, three additional sediment samples were taken for grain size analysis. Sediment samples were dried at 60 °C and grain size was determined with a Malvern Mastersizer using laser diffraction (Buchanan, 1984).

2.3. Laboratory processing

To allow for a correct genus identification of the nematodes under a stereomicroscope, each sediment slice was sieved on a 1000 µm sieve to exclude macrobenthos, and on a 250 µm sieve to retain only the largest nematodes. Therefore, further results presented here are based on the nematodes retained on the 250 µm sieve. All animals and sediments retained on this sieve were stored in -20 °C until further processing.

From both sediment layers of the Control cores (two replicates), 100 nematodes were hand-picked randomly and mounted on slides for identification to genus level under a microscope using the online database NeMys (Vanaverbeke et al., 2015). Investigation of the nematode community structure revealed seven less abundant and four abundant (*Axonolaimus, Metachromadora, Praeacanthonchus and Sphaerolaimus*) genera belonging to different feeding types according to (Moens and Vincx (1997) and Moens et al, (2014): Microvores (Mi); Ciliate feeder (CF); Deposit feeder (DF); Epigrowth feeder (EF); Facultative predators (FP); Predators (Pr) (Table 2).

The ^{13}C stable isotope signal was investigated at the genus level for these abundant genera. As the available biomass was too low for a genus-specific investigation of the isotope signals for the remaining nematodes, they were pooled in an “Other” group. To detect reliable $\delta\ ^{13}C$ in the nematodes tissue, a minimum of 20 µg C per sample was required. Therefore, we picked up 30-35 individuals of *Sphaerolaimus* and between 60-80 individuals for the other genera and the “Other” group from each replicate. The nematodes were hand-picked with a clean needle, counted and rinsed in MilliQ water to remove adhering particles. Nematodes were then transferred to two drops of MilliQ water in 3.5*5.0 mm tin capsules (Elemental Microanalysis Limited). Three blank cups

which contained no nematode were treated in the same way as the sample cups. The capsules were oven-dried overnight at 60 °C, pinched closed and stored in 96 microwell plates in a vacuum desiccator. For each cup, a $\delta^{13}C$ value was measured with a continuous flow isotope ratio mass spectrometer (type Europa Integra) at the UC Davis Stable Isotope Facility (University of California, USA).

Uptake of ^{13}C-labelled diatoms by nematodes is first expressed as specific uptake ($\Delta\delta^{13}C$), which is the difference between the $\delta^{13}C$ of the nematodes at the end experiment (treatments with labelled diatoms) and nematodes from the control treatment (prior to feeding on labelled diatoms): $\Delta\delta^{13}C = \delta^{13}C_{sample} - \delta^{13}C_{control}$ in part per thousand (‰). $\delta^{13}C$ of the sample is calculated as $[(R\ sample - RVPDB) / RVPDB] \times 10^3$ with RVPDB = 0.0112372, the carbon isotope ratio of the Vienna Pee Dee Belemnite standard, and $R_{sample} = [(\delta^{13}C_{sample} / 1000) + 1] \times RVPDB$. Uptake rates of differently sized nematodes cannot be directly compared based on specific uptake, since a larger nematodes absorbing the same amount of ^{13}C as a smaller nematodes would end up with lower $\delta^{13}C$ and $\Delta\delta^{13}C$ (Moens et al., 2014). Hence, we used $\delta^{13}C$ data to calculate total uptake (*I*) according to Middelburg et al. (2000). Total uptake (*I*) in $\mu g^{13}C$ per individual is reflected as excess (above background) ^{13}C and calculated as the product of excess^{13}C (E) and individual organic carbon. E is the difference between the fraction ^{13}C of the sample (F_{sample}) and the control ($F_{control}$): $E = F_{sample} - F_{control}$, where $F = R / (R + 1)$. This total uptake was further standardized and expressed per unit carbon of nematode.

2.4. Data analysis

To test for differences in oxygen penetration depth in the sediment, normality and equality of variances were checked with the Shapiro-Wilks and Levene's test, respectively. Then, a t-test was used for assessing differences between maximum oxygen penetration depth in the Oxic and Hypoxic treatments.

To test for differences in vertical profiles of natural $\delta^{13}C$ values, specific uptake $\Delta\delta^{13}C$ (‰) and uptake of carbon per unit of nematode carbon (µg C µg C^{-1} nematode) among treatments, depth layers and nematode genera, a fully crossed four-factor PERMANOVA based on Euclidean distance was carried out with factors treatment (Tr: Oxic and Hypoxic), depth (De: 0-1 and 1-2 cm) and Genera (Ge: the mentioned nematodes genera and others) as fixed factors, and replicates (Re) nested in treatment, following Braeckman et al. (2011b). Whenever significant differences were observed, pairwise test were performed. Homogeneity of multivariate dispersion ('variance') was tested with PERMDISP for any of the significant terms in PERMANOVA analyses, if significant; this test indicates that observed patterns can be a result of both treatment and dispersion. PERMANOVA analyses were performed using PRIMER v6 with PERMANOVA+ add-on software (Anderson et al., 2008). All other analyses and graphs were performed and drawn with the freely available R 2.14.2 software (http://www.r-project.org). All results are expressed as mean ± standard error.

3. Results

3.1. Environmental variables

The experimental treatment with nitrogen gas resulted in a gradual decrease of the mean oxygen concentration in the overlying water, until hypoxia was reached after 4 hours. Cores remained hypoxic (17.42 % of oxygen saturation) for the entire duration of the experiment. Oxic conditions were maintained in the Oxic treatment throughout the experiment (Table 1).

Table 1. Mean (±se) oxygen concentration (µmol l^{-1}) in the overlaying water during the experiment for Oxic and Hypoxic treatments.

	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**
Oxic	280.00±0.12	283.64±0.05	283.10±0.05	285.00±0.02	286.44±0.12	287.50±0.10
Hypoxic	47.50±0.06	64.00±0.21	60.10±0.31	45.50±0.04	42.00±0.03	39.50±0.04

Oxygen penetration depth was limited to the first centimeter in both treatments at the start and the end of the experiment. There was no significant difference in maximum oxygen penetration depth between the treatments at the start of the experiment ($t = 0.8222$, $df = 4$, $P = 0.4571$). After 6 days, the maximum oxygen penetration depth was significantly lower in the Hypoxic treatment when compared to the Oxic incubation ($t = 5.1237$, $df = 4$, $P = 0.006$, Fig. 3).

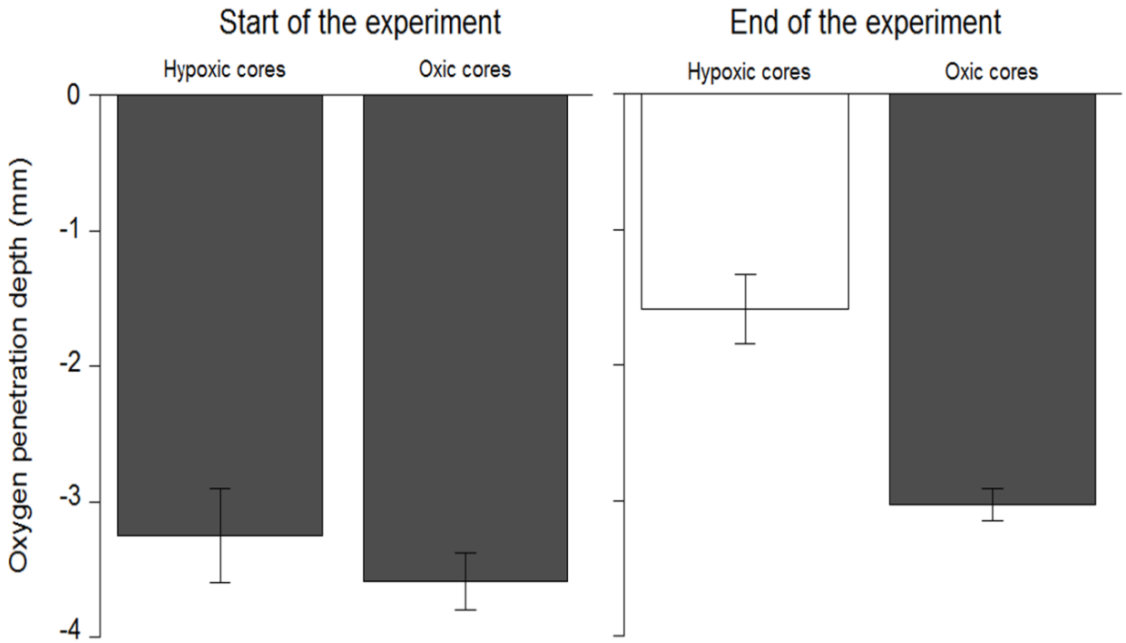


Figure 3. Maximum oxygen penetration depth at the start and the end of the experiment (mean±SE, n=3). The same colour means no significant difference. White bar indicates significantly lower oxygen penetration depth in Hypoxic in comparison with Oxic treatment at day 6 ($p < 0.05$).

3.2. Community structure

The nematode community consisted of 11 genera. The genera *Axonolaimus, Metachromadora, Praeacanthonchus and Sphaerolaimus* were the most abundant accounting together for more than 88.5% of the total nematode abundance in each layer. Deposit feeder and predators were abundant in both layers (Table 2).

Table 2. Relative abundances (%) and feeding type of nematode genera in each layer. Genera in bold font are used for stable isotope analysis. The feeding type classification is based on Moens and Vincx, 1997; Moens et al., 2014). Microvores (Mi); Ciliate feeder (CF); Deposit feeder (DF); Epigrowth feeder (EF); Facultative predators (FP); Predators (Pr).

Genera in 0-1 cm	**Abundances**	**Feeding type**	**Genera in 1-2 cm**	**Abundances**	**Feeding type**
Anoplostoma	2.61	CF	*Anoplostoma*	1.00	CF
Axonolaimus	**9.83**	**DF**	***Axonolaimus***	**13.57**	**DF**
Daptonema	0.52	DF	*Daptonema*	1.53	DF
Metachromadora	**6.82**	**EF**	***Metachromadora***	**10.36**	**EF**
Oncholaimus	4.70	FP	***Praeacanthonchus***	**47.43**	**DF**
Oxystomina	0.52	Mi	***Sphaerolaimus***	**20.51**	**Pr**
Praeacanthonchus	**52.57**	**DF**	*Terschellingia*	2.53	Mi
Sphaerolaimus	**19.27**	**Pr**	*Theristus*	1.03	DF
Terschellingia	1.58	Mi	*Viscosia*	2.04	FP
Viscosia	1.58	FP			
Feeding type contribution (%)			**Feeding type contribution (%)**		
	Mi	2.10		Mi	4.00
	CF	2.61		CF	1.00
	DF	62.92		DF	62.53
	EF	6.82		EF	10.36
	FP	6.28		FP	2.04
	Pr	19.27		Pr	20.51

3.3. Stable isotope signature analysis

The natural $\delta^{13}C$ values of the studied genera varied between -14.33 ± 0.01 and -18.51 ± 0.30 in the first sediment layer. In the second layer, $\delta^{13}C$ values varied between -11.28 ± 4.13 and -16.00 ± 1.75 (Table 3). Other nematode showed the highest values while lowest values were recorded for *Axonolaimus* in both layers. Overall, there was no significant difference in $\delta^{13}C$ natural values among nematode genera and depths (Table 4).

Table 3. Natural $\delta^{13}C$ values mean (±SE) of each genus from control (non-enriched) and treatments (enriched) cores.

		Axonolaimus	***Metachromadora***	***Praeacanthonchus***	***Sphaerolaimus***	**Other**
Control	0-1 cm	**-14.33±0.01**	**-15.32±0.09**	**-15.27±0.05**	**-14.48±0.03**	**-18.51±0.30**
Control	1-2 cm	**-11.28±4.13**	**-13.12±1.45**	**-15.60±0.49**	**-13.09±0.85**	**-16.00±1.75**
Oxic	0-1 cm	-6.65±2.73	-10.47±2.33	-11.17±1.29	-8.89±4.06	-8.46±4.99
Oxic	1-2 cm	-8.86±2.69	-11.58±0.74	-11.46±0.30	-13.75±0.46	-13.62±0.58
Hypoxic	0-1 cm	-10.96±1.51	-11.04±1.81	-10.77±2.32	-9.53±1.89	-12.15±1.16
Hypoxic	1-2 cm	-11.04±1.81	-12.04±0.42	-10.95±1.10	-13.09±0.74	-11.62±3.60

Table 4. Main test results from PERMANOVA analysis for difference in background $\delta^{13}C$ values. P (Perm) = permutation.

	df	**SS**	**Pseudo-F**	**P (perm)**
Depth	1	13.66	2.23	0.338
Genera	4	4.36	0.51	0.738
Depth × Genera	4	0.83	0.02	0.997

After incubation for 6 days with the labelled diatom, $\delta^{13}C$ values in all nematode genera changed in both treatments. $\delta^{13}C$ values of the nematodes in the Oxic and Hypoxic treatments significantly increased compared to the control treatment (Table 3). In all genera, lowest values were found in control but there were no significant differences between Oxic and Hypoxic treatments (Tables 5 and 6). The PERMDISP test was not significant (F = 4.05, P (perm) = 0.053).

Table 5. Main test results from PERMANOVA analysis for difference in background $\delta^{13}C$ values in Control, Oxic and Hypoxic treatments. P (Perm) = permutation.

	df	SS	Pseudo-F	P (perm)
Treatment	2	281,36	24,29	**0,003**
Depth	1	0,42	0.03	0,870
Genera	4	44,57	1,27	0,320
Treatment × Depth	2	32,26	1,00	0,427
Treatment × Genera	8	19,06	0,27	0,962
Depth × Genera	4	13,89	0,25	0,896
Treatment × Depth × Genera	8	30,08	0,29	0,976

Table 6. Main test results from PERMANOVA analysis for difference in background $\delta^{13}C$ values among all treatments. P (MC) = Monte Carlo.

	t	P (MC)
Contro - Oxic	6,20	**0,009**
Control - Hypoxic	6,75	**0,007**
Oxic - Hypoxic	2,45	0,062

Although all genera showed uptake of labelled diatoms, specific uptake was not significantly affected by the Treatment x depth x genera interaction or any other effects (Table 7, Fig. 4).

Table 7. Main test results from PERMANOVA analysis for differences in specific uptake. P (Perm) = permutation.

	df	SS	Pseudo-F	P (perm)
Treatment	1	42.45	1.64	0.252
Depth	1	11.34	0.57	0.491
Genera	4	62.56	1.34	0.292
Treatment × Depth	1	98.22	5.00	0.090
Treatment × Genera	4	48.67	1.04	0.425
Depth × Genera	4	23.15	0.59	0.661
Treatment × Depth × Genera	4	18.49	0.47	0.753

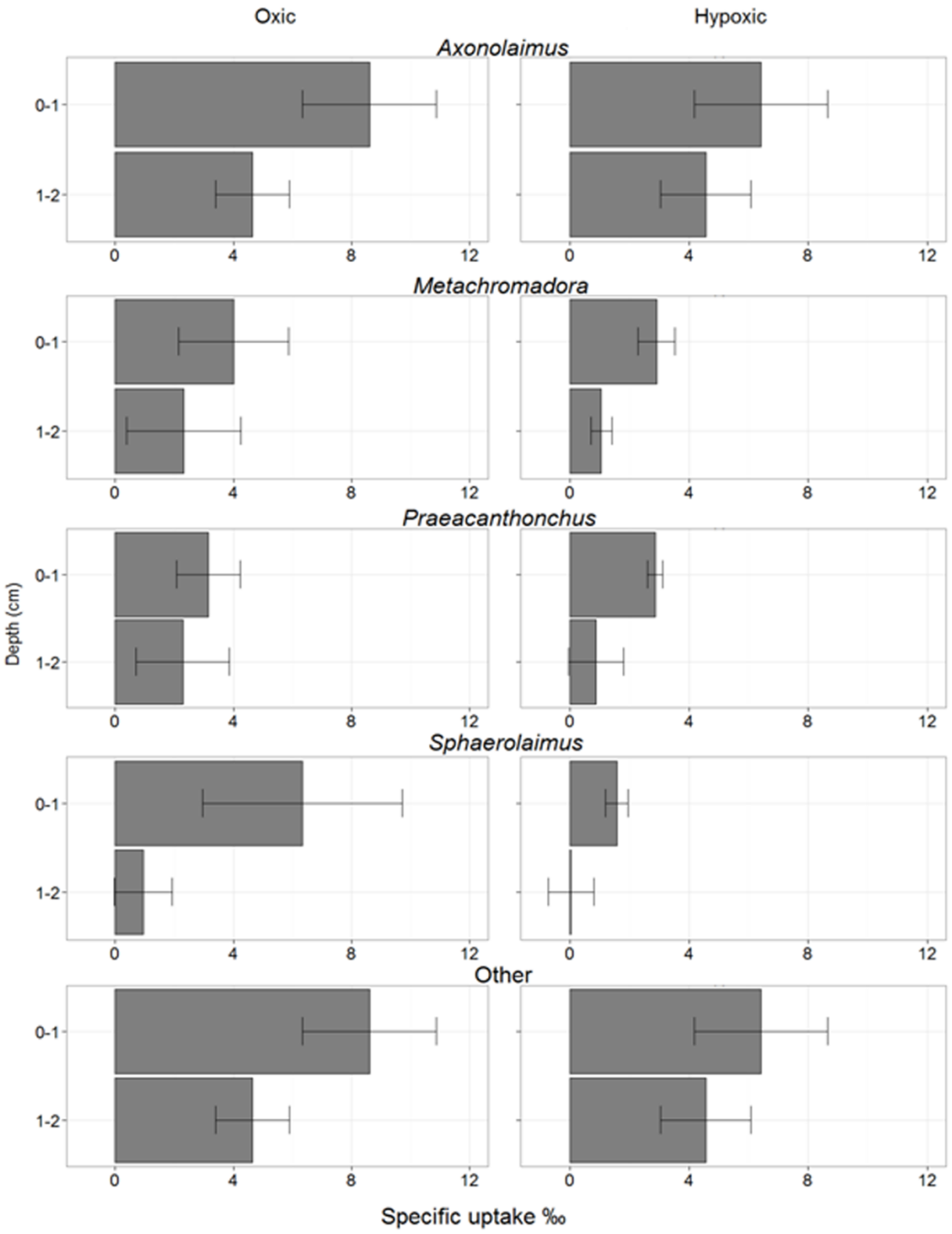


Figure 4. Mean (±SE) specific uptake of labelled diatom ‰ by nematodes under the Oxic and Hypoxic treatments. The same colour means no significant differences.

Finally, uptake of carbon per unit of nematode carbon was not significantly affected by the Treatment x depth x genera interaction or any other interaction or single factor effects (Table 8, Fig. 5).

Table 8. Main test results from PERMANOVA analysis for differences in uptake of carbon per unit of organism carbon (µgC µgC $^{-1}$ nematode). P (Perm) = permutation.

	df	SS	Pseudo-F	P(perm)
Treatment	1	0.00	1.64	0.249
Depth	1	0.00	0.58	0.487
Genera	4	0.00	1.34	0.304
Treatment × Depth	1	0.00	5.00	0.090
Treatment × Genera	4	0.00	1.04	0.417
Depth × Genera	4	0.00	0.59	0.666
Treatment × Depth × Genera	4	0.00	0.47	0.758

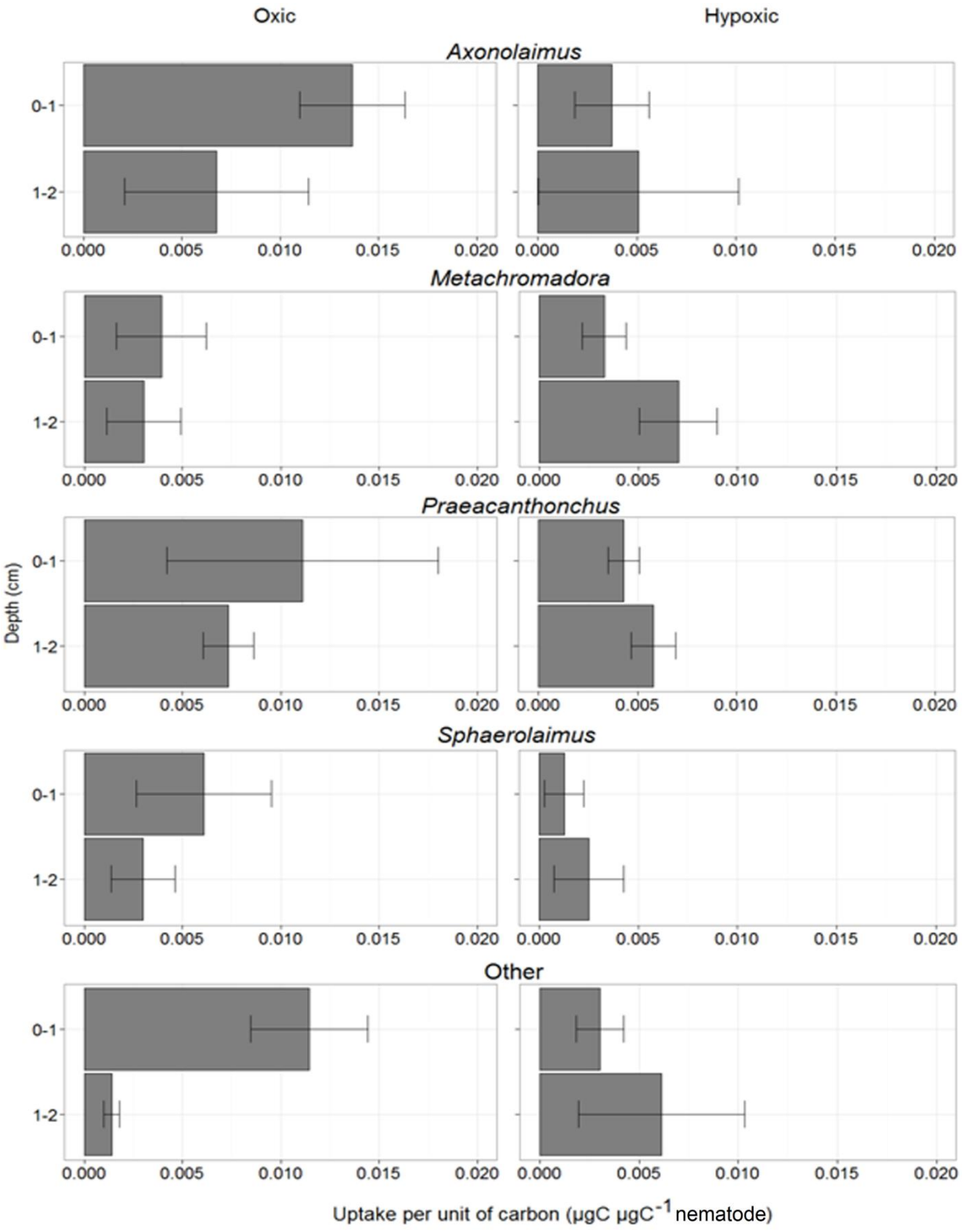


Figure 5. Mean (±se) uptake of carbon per unit of organism carbon (µgC µgC $^{-1}$ nematode) under the Oxic and Hypoxic treatments. The same colour means no significant differences.

4. Discussion

Our results showed a low carbon uptake by all genera suggesting that the added diatoms only represented a limited food source for the studied nematode genera. In addition, the present study showed that short-term hypoxia (6 days) had no effect on the feeding activity of the abundant nematode genera and the remaining bulk community (other nematodes). The reasons for this low carbon uptake are discussed below.

4.1. Sediment oxygen penetration depth under induced hypoxia

In the present study, permanent hypoxia was successfully created in the overlaying water throughout the experiment (Table 1). The maximum oxygen penetration depth in the Oxic treatment was almost similar as in previous laboratory incubations in very fine sand (till 2.5 mm, Steyaert et al., 2005) and muddy sediment (till 2.6 mm, Van Colen et al., 2008, 2012; and 3.7 mm, Braeckman et al., 2014). A significant upward migration in the oxygen penetration depth of 1.5 mm was observed after 6 days of the hypoxic incubation reflecting the consequences of a water-column process on the benthic environment as observed in earlier experiments (Taheri et al., 2014).

4.2. Consumption of added diatom carbon by nematodes

We observed uptake of labelled diatoms in nematodes in the entire 0-2 cm of sediment, although we added labelled diatoms only on the top of sediment. Nematodes from the 1-2 cm consumed as little labelled diatoms as the nematodes from the upper cm layer. This can either be explained by downward mixing of the labelled food to deeper layers where it is consumed, or by vertical migration of nematodes towards and away from the

sediment-water interface. Downward transfer of food by advective currents has been observed in permeable sediments (Huettel and Gust, 1992), but not in finer sediments as incubated in the current experiment. In addition, advective flows were not induced in our experimental set-up. In a laboratory experiment, Braeckman et al. (2011a) also indicated that downward transfer of labelled diatom by macrofaunal activity was not very pronounced on short time scales. However, vertical transport of fresh detritus by macrofauna was observed in a field experiment (Graf, 1989). According to our visual inspections during the incubations and after sieving, large macrofauna was absent in our cores. Therefore, we attribute the labelling of the nematodes in the second cm layer to vertical migration towards the sediment-water interface, followed by a downward migration to the deeper sediment as observed before in subtidal sediments (Franco et al., 2008b).

There was no significant difference in $\delta^{13}C$ background values among nematode genera and depths although the $\delta^{13}C$ signal in the “other” nematodes was more negative (Table 3). This group comprised 11.51 and 8.13 percent of the whole nematode community in 0-1 and 1-2 cm depth, respectively. However, the natural carbon isotope signatures of all studied genera and other nematodes overlap with the range of previous studies on the same genera in the Westerschelde estuary (Moens et al., 2002; Moens et al., 2005; Steyaert et al., 2007; Moens et al., 2014) which in turn is in the same range as of microphytobenthos (-14 till -21‰) in the Schelde Estuary (Moens et al., 2002; Moens et al., 2005) or other intertidal areas (till -21 ‰; Vafeiadou et al., 2014). It suggests that microphytobenthos is an important source for intertidal flat nematodes (Moens et al., 2002; 2014). However, the overall low C uptakes in our study suggest that the added

diatoms constituted a limited addition to the diet of the investigated nematode communities. In the following paragraphs, we discuss different possibilities which may explain this low uptake. We explore three possible reasons: 1) the nematodes feed preferentially on the other, non-labelled diatoms present in the experimental cores 2) the labelled diatom was not really available to the nematodes, and 3) the nematodes were not active during the experiment.

Feeding preference and selectivity of nematodes on benthic diatoms (e.g. Tietjen and Lee, 1973; Blanchard, 1990; Moens et al., 1999a) and bacteria (e.g. Moens et al., 1999b) has been reported in different studies. The shape of the diatom species added in this experiment (prolate spheroid with two spins like cylinders; Hillebrand et al., 1999) may not attract nematodes to graze on it. It is possible that the nematode community in our experimental cores is adapted to feed on the other available, non-labelled diatoms in the sediment (Moens and Vincx, 1997; Franco et al., 2008b; Braeckman et al., 2011b) which were better accessible with their mouth shape and size.

In the present experiment the added diatom carbon represented 0.65% of the total organic carbon in the first cm of the sediment. It is possible that the labelled diatom was not really available to the nematodes due to the dilution in the sediment organic matter pool; therefore nematodes had more chance to feed on the other abundant diatoms. Moens et al. (2002) indicated higher feeding actively by nematodes when added algal carbon represented 1-2 % of the total sediment organic carbon. This higher relative availability (in comparison with present study) can increase the probability that nematodes do feed on the additional food.

A final explanation for the low uptake is the fact that nematodes were not active during the experiment. This can be either as the result of sediment sampling and its effect on nematodes (stress) or the effect of oxygen stress on the feeding activity. Taheri et al. (2014) showed that experimental sediment handling did not affect nematode community characteristics. In addition, nematode communities were shown to be tolerant to short-term oxygen stress both in the intertidal (Steyaert et al., 2005) and subtidal (Taheri et al., 2014, 2015) areas. Steyaert et al. (2007) also indicated an active feeding on labelled diatom by some nematodes during 14 days anoxia. According to the low uptake even in the Oxic treatment, we can conclude that the low uptake of labelled diatoms was not a result of inactivity of nematodes during our experiment. Therefore, it can be conclude that the low carbon uptake could be related to both low feeding preference of nematodes and low availability of added diatoms in our experimental cores. Therefore, it is possible that the low uptake does not allow a firm interpretation of the results obtained by this experiment. However, none of the investigated genera and the remaining “other” nematodes showed a reduced feeding after 6 days of Hypoxic treatment. It should be noted that oxygen penetration depth was already limited to the upper half cm before the onset of the experiment. Hence, most nematodes probably adapted to live in oxygen stressed environment. The absence of a negative effect of hypoxia on nematode feeding activity could be related to their ability to use minimum oxygen concentrations in their environment as *Axonolaimus, Metachromadora and Sphaerolaimus* have been reported as tolerant genera for oxygen stress (Steyaert et al., 2007; Muresan, 2012). However, our results are in contrast to earlier observations, where nematodes from a similar environment did show a reduced feeding activity after 2

weeks of experimentally induced hypoxia/anoxia (Steyaert et al., 2007). This could be related to i) stress in the nematode communities as a consequence of sediment manipulation (mixing and wet sieving) in the mentioned experiment (Steyaert et al., 2007), ii) species-specific response of nematode community to oxygen stress and natural adaptations of each species (Modig and Olafsson, 1998; Wetzel et al. 2001; Steyaert et al., 2007), iii) longer incubation time (14 days) in comparison with our study, or iv) they reported presence of sulphide (13-31 µmol l^{-1}) in the 2 week anoxic treatment in the experiment. Such development is unlikely to have occurred in our experiment as it has been demonstrated that more than 2 weeks of constant low oxygen concentration (<32 µmol l^{-1}; Gray et al., 2002) is needed before the onset of a sulphide flux to the overlying water can be observed (Kristiansen et al., 2002).

Our results of total uptake of C per unit of carbon of organism (µgC $µgC^{-1}$ nematode) are in the lower range as reported for other nematode communities from fine sediments (Franco et al., 2008b; Pasotti et al., 2012) that are not subjected to oxygen stress, again indicating that uptake of food is not reduced by oxygen stress. Therefore, the reduction in nematode respiration rates in hypoxic conditions (Ott and Schiemer, 1973; Braeckman et al., 2013) could be related to a reduced nematode activity, or to a switch to a partly anaerobic metabolism (Wieser and Kanwishe, 1961). However, our experiment does not allow making clear statements on the actual underlying reasons.

Our experiment provides information on the C-uptake of four abundant genera. Diatoms are known to be an important food source for *Axonolaimus* (DF), *Praeacanthonchus* (DF) and *Metachromadora* (EF) (Moens and Vincx, 1997; Moens et al., 2005; 2014). *Sphaerolaimus* is a large predator nematode with a large and heavily cuticularized

mouth (Moens and Vincx, 1997; Rzeznik-Orignac et al., 2008; Vafeiadou et al., 2014). Uptake of labelled carbon by this genus could be related to predation on the other diatom feeding nematodes or organisms or non-specific ingestion of labelled cells or labelled cell contents whilst moving or searching for prey (Moens and Vincx, 1997; Rzeznik-Orignac et al., 2008; Vafeiadou et al., 2014). However, it has been demonstrated that many marine nematodes may be opportunistic feeders and adding fresh food triggers opportunistic responses, for all of the nematodes, independent of their classification which may change strategies (some plasticity) in their feeding behaviour in response to available food (Moens and Vincx, 1997; Moens et al., 2014).

5. Conclusion

The low carbon uptakes by all studied genera hamper interpretation of the obtained results in this experiment. The added diatoms represented only a limited food source for nematodes as discussed before. However, our results suggest that feeding activity by the larger intertidal nematodes was not significantly affected by hypoxia in the overlaying water column. This result can be supported by the lack of negative effect of short-term hypoxia/anoxia on density and diversity of different coastal nematode communities (Guerrini et al., 1998; Steyaert et al., 2005; Taheri et al., 2014; 2015) though further studies are needed in order to better understand the effect of oxygen stress on the nematode feeding activity.

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Chapter 5 General discussion and future challenges

The general objective of this PhD research was to investigate the effect of oxygen depletion (severity and duration) in the water column on the marine free-living nematodes living in coastal sediments. This was achieved by means of a combination of *in situ* and laboratory experiments in a variety of environmental settings that provided insight in the response of different nematode communities and their feeding activity to short and long-term oxygen stress, and subsequent recovery processes.

The three specific objectives, and their main results, can be formulated as follows (Fig. 1):

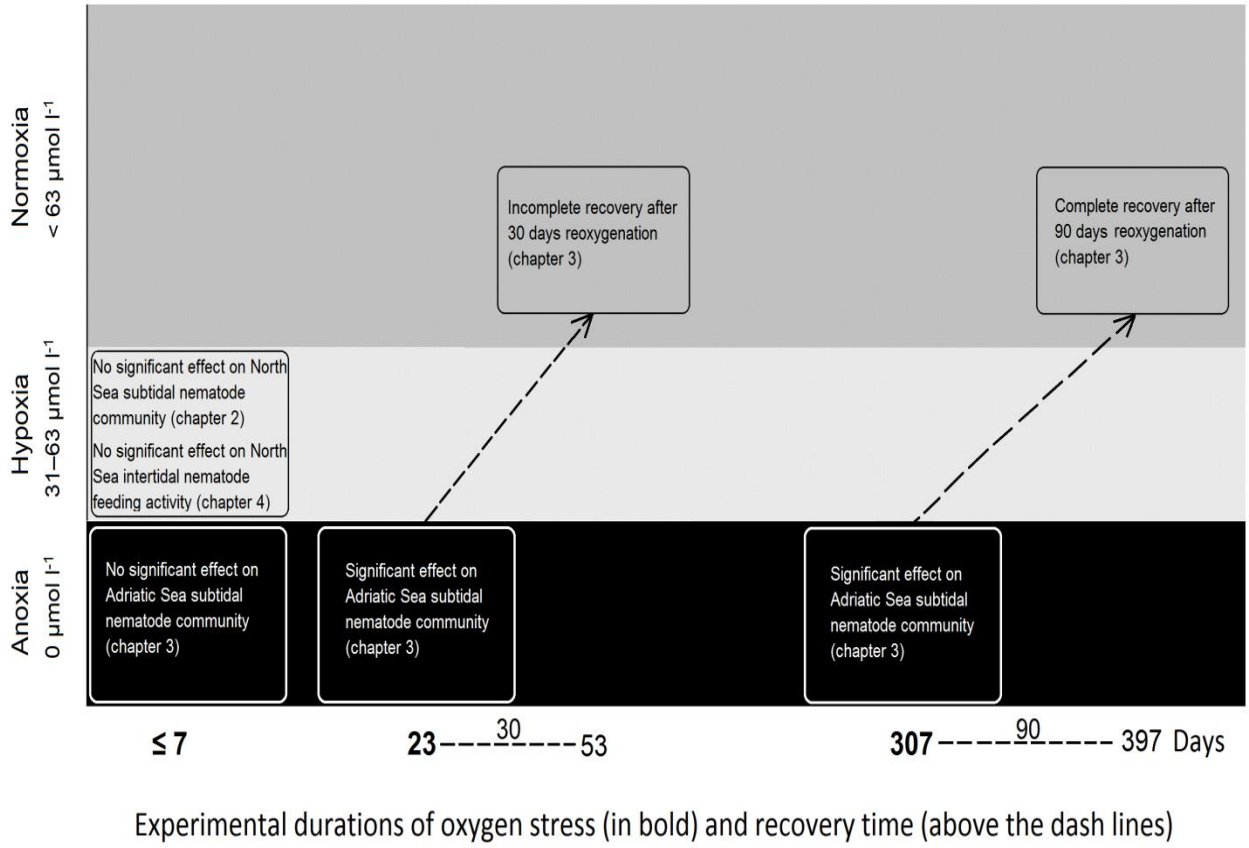

Figure 1. Schematic overview of the response of nematode communities' characteristics and feeding activity to oxygen stress (severity and duration) which were obtained from the present PhD thesis.

- **To evaluate and compare the effect of short-term hypoxia (1 and 7 days) on structural characteristics (density, diversity, vertical distribution and community structure) of different nematode communities sampled from different sediment types subjected to similar global environmental settings (temperature, timing and magnitude of phytoplankton bloom).**

Our results indicated that nematode communities were different among the different stations. However, independent of the sediment types and nematode community structures, short-term hypoxia had no significant effects on density, species diversity, community structure and vertical distribution of nematodes. It means that nematode communities are tolerant to short-term hypoxia events.

- **To evaluate the response of nematode communities to short and long-term anoxia, and the subsequent recovery of the nematodes communities.**

Our results confirmed that the nematode community characteristics (density, diversity, community structure and vertical distribution) were not affected by short-term anoxia while total density and vertical distribution, species richness and diversity decreased in longer periods of anoxia (23 and 307 days). In addition, functional aspects (based on feeding type contributions) changed when anoxia lasted longer (23 and 307 days). The nematode community showed a species-specific response to the duration of anoxia. We observed that 21 species even survived 307 days of anoxia. Our results also showed that total nematode density, Shannon diversity and evenness of communities exposed to 23 days of anoxia did not recover after 30 days sediment reoxygenation while nematode species richness increased and epistrate feeding nematodes reappeared in

the community. A full recovery in te nematode community was observed after a recovery phase of 90 days after a 307 days anoxia.

- **To evaluate the effect of short-term hypoxia (6 days) on the feeding activities of the intertidal nematodes**

As shown in chapter 4, the low carbon uptake by all studied genera hampers the interpretation of the results obtained in this experiment. The added diatoms represented only a limited food source for nematodes as discussed before. However, our results suggest that feeding activity by the larger intertidal nematodes was not significantly affected by hypoxia in the overlaying water column. The low carbon uptake might be related to low access of nematodes to the added diatoms, in combination with a low feeding preference for the added diatoms in the experimental cores.

In this chapter, the results of the present thesis are integrated and compared with available data regarding oxygen stress in marine free-living nematode communities. First, the effects of short-term hypoxia and anoxia (less than 7 days) to both structural aspects of nematode communities (density, diversity, vertical distribution) from different sediment types, and their feeding activity is discussed. Second, we focus on the response of nematode communities' characteristics to longer term anoxia (23 and 307 days) with emphasis on tolerant nematode species. Third, recovery patterns of nematode communities are discussed based on results derived from this thesis and the literature (Fig. 1). In addition, a comparison with the effect of hypoxia/anoxia on the meiofaunal and macrofaunal communities of the same experimental site and other

areas was performed. Finally, some future challenges regarding investigations on the impact of oxygen on marine free-living nematode communities are suggested.

5.1. The effect of short-term hypoxia and anoxia (≤7 days) on nematode communities

The nematode community structure within a certain area is shaped by the regional taxon pool, their adaptation abilities to environmental conditions and abiotic/biotic interactions. The nematode communities generally reflect differences in sediment type (Vanaverbeke et al., 2003). In coarser sediments with higher permeability (median grain size >200 mm), the pore water flow can reach to deeper sediment layers, providing more oxygen in the sediment (Vanaverbeke et al., 2011). As nematode communities in this coarser sediment are naturally adapted to live in continuously oxygenated conditions, it is expected that nematodes from this sediment type are more sensitive to oxygen stress than nematode communities from finer-grained sediments where oxygen concentrations are markedly lower, or where oxygen is absent. Therefore, in chapters 2, 3 and 4, combined *in situ* and laboratory studies were conducted to investigate the potential effect of short-term hypoxia/anoxia conditions on nematode communities (density, diversity, vertical distribution) and feeding activity from different sediment types and ecosystems.

In our study areas, the range of temperature and salinity in the Belgian part of the North Sea (BPNS) (5-20ºC and 32-34, respectively, Provoost et al., 2013) and in the Gulf of Trieste, in the northern Adriatic Sea, (8-20 ºC and 36-38, respectively, Faganeli et al., 1985) are somewhat close to each other. The most important differences between these two ecosystems are water circulation and oxygen depletion. The seawater at the BPNS

is continuously moving because of the prevailing tidal currents and the wave actions (Degraer et al., 2006); therefore it is oxygenated. Due to local topography, water residence time at the sea surface of the Adriatic Sea is about 250 days (Poulain and Hariri, 2013) and the area undergoes seasonal oxygen depletion events (Malej and Malacic, 1995; Giani et al., 2012). In this study, investigating both the North Sea and the Adriatic Sea enabled us to make some generalizations about the response of nematode communities to short-term hypoxic/anoxic conditions regardless of the variability in their environments.

Our findings indicate that nematode communities are generally tolerant to short-term hypoxia/anoxia conditions, independent of species composition, sediment type and geographical location (Table 1). Our results are in agreement with earlier studies (Guerrini et al. (1998) and Steyaert et al. (2005)) which showed a tolerance of nematode communities to short-term (≤ 1 week) anoxia. These results however are in contrast with Steyaert et al. (2007) and Arroyo et al. (2012). Apart from different nematode communities in all studies and possible species-specific responses, this could also be explained by methodological differences between their studies and our work: in the first study, nematodes from the upper 2 centimeter which are more sensitive to oxygen stress (Jensen, 1984) were examined. This nematode community was dominated by 16 species including *Daptonema setosus*, *D. tenuispiculum*, *Chromadora macrolaima* and *Paracanthonchus heterodontus*. A full mortality was observed for half of the dominant species and the density of 3 species decreased sharply. In addition, the sediment was mixed and manipulated which could have caused stress on the nematode communities before the onset of the experiment. However,

hydrogen sulphide was not produced at the end of the 14 days incubation. In the second study, details on the nematode community were not mentioned in the paper. Here, a drifting algae mat was used for creating hypoxia. Change in pH and temperature, development of sulphidic conditions (toxic compound) and the accumulation of ammonia acting in combination with hypoxia, can be possible reasons for the observed decrease in meiofaunal densities during a 6 days study.

Table 1. Dominant nematode species (> 50% of the total abundance) in our study sites in different sediment types. * = median grain size was not measured.

Range of the sediment size	**Dominant species**
41.62 ±17.90 µm (North Sea):	*Ascolaimus elongatus, Oncholaimellus calvadosicus, Sabatieria breviseta, Sabatieria elongata* and *Sabatieria punctata*
165.90 ±20.07 µm (North Sea):	*Paramonhystera longicaudata, Sabatieria breviseta, Sabatieria celtica, Sabatieria punctata* and *Microlaimus conothelis*
350.89 ±42.48 µm (North Sea):	*Epsilonema serrulatum, Paracyatholaimoides asymmetricus, Prochromadorella attenuata, Rhynchonema megamphida* and *Xyala riemanni*
*Silty sand (Adriatic Sea):	*Theristus longissimecaudatus, Terschellingia longicaudata, Calomicrolaimus compridus, Metalinhomoeus effilatus* and *Spilophorella euxina*

Morphological, behavioural and physiological adaptations help marine nematodes to tackle short-term hypoxic/anoxic conditions. Upward migration to the water column (Wetzel et al., 2001) or surficial sediment layers was not observed in our experiments (chapters 2 and 3), which confirms nematode resistance to oxygen stress. It has been demonstrated that long and slender nematodes are more adapted to live in suboxic sediments (Soetaert et al., 2002). Their higher length/width ratio results in higher oxygen absorption efficiency per unit of volume, a higher length let for faster migration between suboxic and oxic layers and could allow bridging the gap between oxic and

anoxic spots in the sediment (Soetaert et al., 2002). A decrease in respiration rate (metabolism) even after 24 hours (Braeckman et al., 2013) has also been reported as a mechanism to overcome hypoxic/anoxic conditions (Ott and Schiemer, 1973).

In contrast to several studies about the effects of hypoxia/anoxia on the nematode communities (density, diversity, vertical distribution), little is known about their feeding ecology in oxygen stressed conditions (Steyaert et al., 2007). All aerobic organisms need oxygen to survive and grow. Nematodes absorb oxygen by diffusion from the environment; therefore oxygen availability in the surrounding area has a key role in the metabolism of nematodes (Braeckman et al., 2013). A decrease in feeding activity of some copepods (De Troch et al., 2013) and intertidal nematodes in hypoxic/anoxic conditions (Steyaert et al., 2007) was reported before. In Chapter 4, the effect of overlaying water hypoxia (6 days) on the feeding activity of intertidal nematodes was studied. The results of this chapter showed low carbon uptake by all studied genera which hamper the interpretation of the results. The added diatoms represented only a limited food source for nematodes as discussed before. However, our results suggest that feeding activity by the larger intertidal nematodes was not significantly affected by hypoxia in the overlaying water column which is in contrast with Steyaert et al. (2007).

In this thesis, two different methods were used to create short-term oxygen stress. In the second and fourth chapters, hypoxia was created by bubbling the overlaying water with nitrogen gas. A fast decline in the oxygen concentration of the overlaying water was observed, reaching hypoxia after 4 hours (chapter 4). In chapter 3 (in situ chamber incubation), the oxygen concentration decreased more gradually so that hypoxia and anoxia were reached only after four and seven days, respectively (Metzger et al., 2014).

From these experiments, we can conclude that marine benthic nematodes are also tolerant to even fast changes in oxygen concentration.

Oxygen stress (hypoxia/anoxia) can cause a mass mortality and a reduction in total biomass of macrofauna (Van Colen et al., 2008; Riedel et al., 2012). In general, macrofauna are more vulnerable than meiofauna to hypoxic and anoxic conditions (Levin et al., 1991; Van Colen et al., 2009). To investigate the effect of short-term hypoxia and anoxia on macrofauna, a comprehensive study was carried out with eleven deployments of underwater camera and sensor equipped benthic Plexiglas chambers at 24 m depth in the Gulf of Trieste, Northern Adriatic Sea (the same location as our experiment). The results indicated species-specific responses of macrofauna (epi and infauna) to hypoxia and anoxia. In experimental chambers, a gradual decrease in oxygen concentration from normoxia to hypoxia (after 1.5 days) and further to anoxia (after 3 days) was observed. Mortality (14 species from 40) in macrofauna community started at hypoxia when dissolved oxygen concentrations reached < 0.5 ml l^{-1}. Thirteen more species further died exclusively during anoxia. Most species died before the onset of hydrogen sulphide (H_2S) production. Survival rates were highest among mollusks, anthozoans and ascidians while most polychaetes, decapods and echinoderms experienced mortalitiy in the experiment. Epifauna and mobile forms were more vulnerable than infauna and sessile forms, and predators more sensitive than deposit-feeders and suspension-feeders to oxygen stress. As Foraminifera (Langlet et al., 2013), harpacticoid copepods (Grego et al., 2014) and nematodes (Taheri et al., 2015) were investigated in the same sampling site with the same methodology (experimental chamber) and global environmental conditions (salinity, sediment type, depth and

gradual decrease in oxygen concentration), we are allowed to compare how different benthic faunal group (macrofauna and meiofauna) are affected by short-term hypoxia and anoxia. As is mentioned in chapter 3, copepods were the most sensitive group to short-term hypoxia and anoxia (5 days), followed by nematodes and Foraminifera. Comparison (based on mortality of species) between these two benthic groups showed a higher resistance of meiofauna especially nematodes and Foraminifera to short-term oxygen stress when compared to macrofauna (Riedel et al., 2012; Langlet et al., 2013; Taheri et al., 2015).

In total, our results indicated that short-term hypoxia/anoxia conditions did not affect density, species diversity, community structure, vertical distribution and feeding activity of coastal nematodes in different sediment types separately. However in a large scale like BNPS with different sediment types and biogeochemistry (Braeckman et al., 2014), accumulation of deposited organic matter in the sediment after phytoplankton blooms can change the nematode community, density and diversity (Vanaverbeke et al., 2004; Franco et al., 2008a). Decomposition of organic matter can create anoxic patches on the sediment (Gambi et al., 2009) and consequently can negatively affect the nematode density and change community (Franco et al., 2008a; Gambi et al., 2009; Ferreira et al., 2015). Our results showed the highest values for diversity and evenness indices in the sediment with the lowest chlorophyll content (station 330) while the lowest values of these indices were observed in the sediment with the highest chlorophyll content (station 700). The low oxygen availability in finest sediment (with highest chlorophyll content) may have favored for development of communities dominated by tolerant

genera (like *Sabatieria* at stations 700 and 115 bis) and, consequently, decreasing relative densities of all other species after phytoplankton blooms.

5.2. The effect of longer term anoxia (23 and 307 days) on nematode community

Aquatic nematodes show a wide range of tolerance to oxygen deficiency. Their response is species-specific and related to the duration of oxygen deficiency (Modig and Olafsson, 1998; Travizi, 2000; Wetzel et al., 2001; Muschiol et al., 2015). It is reported that some nematode species are able to live in hypoxic and anoxic conditions temporarily (Wieser and Kanwisher, 1961; Braeckman et al., 2011b; Muresan and Gomoiu, 2012; Sergeeva and Zaika, 2013). In chapter 3, we focus on the effects of longer term anoxia (23 and 307 days) on the nematode communities. Our results showed a sharp decline in density, species richness and diversity at 23 and 307 days anoxia which is in agreement with the other studies (e.g. Moodley et al., 1997; Modig and Olafsson, 1998; Wetzel et al., 2002; Van Colen et al., 2009). However, 21 nematodes species including adults and even gravid females were still alive after 307 days anoxia. Some of them have been previously reported as being tolerant to oxygen stress in different ecosystems (Table 2).

Table 2. Surviving nematode species at 307 days anoxia in our study in the Adriatic Sea and in the other studies with more than two months hypoxia/anoxia periods. NA = data not available.

Genera	Species	Literatures
	Axonolaimidae	
Odontophora	*O. fatisca*	Wieserand Kanwisher, 1961
		NA
	Camacolaimidae	
Camacolaimus	*C. tardus*	Modig and Olafsson, 1998, Muthumbi et al., 2004

	Chromadoridae	
Actinonema	*A. fidatum*	NA
	A. pachydermatum	NA
	Comesomatidae	
Sabatieria	*S. celtica*	Gambi et al., 2009, Muresan and Gomoiu, 2012
Setosabatieria	*S. hilarula*	Travizi and Vidakovic, 1994
	Cyatholaimidae	
Nannolaimoides	*N. decoratus*	NA
Marylynnia	*M. complexus*	NA
	Desmoscolecidae	
Tricoma	*Tricoma* sp.1	NA
	Leptolaimidae	
Antomicron	*Antomicron* sp.1	NA
Halaphanolaimus	*H. harpaga*	NA
Leptolaimoides	*Leptolaimoides* sp.3	NA
	Linhomoeidae	
Metalinhomoeus	*M. effilatus*	Muresan and Gomoiu, 2012, Sergeeva and Zaika, 2013
Paralinhomoeus	*P. caxinus*	Travizi and Vidakovic, 1994, Sergeeva and Zaika, 2013
Terschellingia	*T. longicaudata*	Van Gaever et al., 2009, Vanreusel et al., 2010
	Oncholaimidae	
Viscosia	*V. elegans*	Gambi et al., 2009, Muresan and Gomoiu, 2012
	Selachinematidae	
Richtersia	*R. staresensis*	Muresan and Gomoiu, 2012
	Xyalidae	
Daptonema	*D. lata*	Modig and Olafsson, 1998, Muthumbi et al., 2004
	Daptonema sp.1	Modig and Olafsson, 1998, Muthumbi et al., 2004
Promonhystera	*Promonhystera* sp.1	NA
Theristus	*Theristus* sp.1	Sergeeva and Zaika, 2013

Apart from the effect of anoxia, the decline in nematode density after 23 days anoxia could be related to the presence of H_2S and food limitation (see chapter 3). In short, the epistrate feeder nematodes (*Calomicrolaimus compridus* and *Spilophorella euxina*) disappeared from the group of dominant species after 23 days anoxia. Diatoms are an important food source for epistrate feeder nematodes (Moens et al., 2014). Probably diatom density in this treatment decreased, due to a lack of light at 24m depth in combination with settling sediment and large animals on the outer surface of the experimental chamber (Grego et al., 2014; Metzger et al., 2014). The proportion of the selective and non-selective deposit feeders was less affected in this treatment. The degradation of the dead macro-infaunal community (Metzgeret al., 2014) increased the amount of organic matter in this treatment, and as a consequence the abundance of bacteria probably increased (de Moraes et al., 2014; Gallizia et al., 2005). Bacteria are an important food item for both selective (Moens and Vincx, 1997; Neira et al., 2013) and some non-selective deposit feeders (Ingels et al., 2011). Therefore we assume that they were not food limited. A recent study indicated that bacterivorous nematodes could survive long term hypoxia (one year) as long as the microbial food source was available (Muschiol et al., 2015). However, more studies are needed to better understand the combined effects of food scarcity and oxygen stress on the nematode communities.

Metzger et al. (2014) indicated that H_2S concentration was not very high in the 307 days anoxia treatment. Therefore, the high mortality and sharp decrease in species richness of living nematodes after 23 days anoxia could be mainly related to the direct effect of anoxia. *Metalinhomoeus effilatus* a non-selective deposit feeder nematode was dominant among the surviving nematodes. Probably, being able to explore a wide range

of food sources including bacteria and even dissolved organic carbon (Moens and Vincx, 1997; Moens et al., 2014) in combination with some kinds of adaptations help this species to survive in long-term anoxia (Muresan and Gomoiu, 2012; Sergeeva and Zaika, 2013).

Like the other aerobic organisms, most aquatic nematodes need oxygen to complete oxidation of food in the aerobic metabolism. Some recent studies showed that marine nematodes cannot survive and grow as well as cannot complete their life cycles in permanent anoxia (Danovaro et al., 2010; Bernhard et al., 2015; Urkmez et al., 2015). The survival of 21 nematodes species after 307 days anoxia is in contrast with the mentioned studies. As it is mentioned in Table 2, some nematodes tolerant to 307 days anoxia have previously been reported in different marine ecosystems with more than two months of oxygen stress. Our results did not show any exact/specific adaptation mechanisms of the surviving nematodes to long-term anoxia (307 days). However, several studies indicated morphological, behavioural or physiological adaptations to successfully cope with anoxic conditions. Generally, long and slender nematodes are more adapted to live in hypoxic/anoxic sediment (Soetaert et al., 2002; Braeckman et al., 2013). Their higher L/W ratio results in higher oxygen absorption efficiency per unit of volume, while longer nematodes have more chance to bridge oxic spots with their body (Soetaert et al., 2002). Our results showed both short worms with a short conical tail (*Tricoma* and *Richtersia staresensis*) and long worms with an elongated tail (*Metalinhomoeus effilatus* and *Terschellingia longicaudata*) among the tolerant nematode species which means that the surviving nematodes used the other adaptation mechanisms besides body size to cope with the anoxia.

Based on the morphology of the buccal cavity, all four feeding types were observed in the nematode community in the 307 days anoxia treatment. This may indicate that the ability to tolerate anoxia was not related to feeding type and hence the trophic structure of the nematode community (Wieser and Kanwisher, 1961). In some aquatic nematodes like *Terschellingia*, a decrease in respiration rates as an adaptation mechanism to cope with oxygen stress has been reported (Ott and Schiemer, 1973; Braeckman et al., 2013). Although respiration rates were not measured in the present study, it is possible that our studied genera and species decreased their respiration rates as well. *Sabatieria celtica* and *Terschellingia longicaudata* are two other nematodes which have been recognized as tolerant species to long-term anoxia (307 days). Nicholas et al. (1987) observed some dark and dense intracellular inclusions mostly within the intestinal cells of *Sabatieria wieser* and *Terschellingia longicaudata* which were collected from anoxic sediment. The same structure was reported in a limnetic nematode *Tobrilus gracilis* in hypoxic/anoxic sediments as well (Nuss, 1984). It is possible that these intracellular inclusions help nematodes with the detoxification of harmful substances like sulphide (Nicholas et al., 1987) or allow nematodes (*Tobrilus gracilis*) to shift to an anaerobic metabolism in anoxic condition (Schiemer and Duncan, 1974).

With regard to the short life span for most marine nematodes (Platt and Warwick, 1988) as well as the occurrence of gravid females in the community after 307 days anoxia (Chapter 3), a continued reproduction after long-term (307 days) anoxia can not be excluded. Oxygen is necessary for the formation of cuticular and collagenous material and the reproduction and the development of juveniles of most marine nematodes (Jensen, 1995, Giere, 2009). Ovoviviparity for a few marine nematode species

(*Geomonhystera disjuncta*, Van Gaever et al., 2006; *Metachromadora vivipara*, Steyaert at al., 2007; Vanreusel et al., 2010) was reported as an adaptation to life in anoxic and sulphidic sediments. Ovoviviparity was not observed in gravid females in our experimental nematode community after 307 days of anoxia, which means that the surviving nematodes used the other adaptation mechanisms, or a combination of them to cope with the oxygen stress. Benthic foraminifera are now known as a tolerant meiofauna group to anoxia in marine sediments (Giere, 2009; Langlet et al., 2013; Nomaki et al., 2015). It is reported that foraminifera species are able to use nitrate as an electron acceptor in their respiration under hypoxic and anoxic conditions (Piňa-Ochoa et al., 2010; Bernhard et al., 2012). Little is known about the metabolism of nitrogen in ectosymbiotic bacteria of marine nematodes so far, though it has been reported for at least two marine nematode species *Stilbonema* sp. and *Laxus oneistus* (Hentschel et al., 1999). The other adaptation mechanisms including the ability to switch between aerobic and anaerobic metabolisms (*Tobrilus gracilis*, Schiemer and Duncan, 1974; Barbercheck and Duncan, 2004; Tahseen, 2012) and symbiosis with sulfur-oxidizing bacteria in *Astomonema* (Giere et al., 1995; Musat et al., 2007) and *Parastomonema* (Kito, 1989) have been reported in certain aquatic nematode species in oxygen stressed environments. However, more studies are needed to better understand different nematode adaptations to oxygen stress.

Although nematodes are the most abundant and diverse meiofauna group in the OMZs (e.g. Cook et al., 2000; Neira et al., 2001; Veit-Köhler, et al., 2009; Guilini et al., 2012; Singh and Ingole, 2016), their density and diversity is much lower (e.g. Guilini et al., 2012; Singh and Ingole, 2016) than in most coastal areas (e.g. in the present study

chapter 2: 205 species and chapter 3: 154 species) and other deep-sea environments (e.g. Guilini et al., 2012; Singh and Ingole, 2016). Nematode assemblages in OMZs are characterized by a low diversity with some species/genera being tolerant to low-oxygen conditions (e.g. Levin, 2003; Guilini et al., 2012; Singh and Ingole, 2016). Exposure history plays an important role in the potential of marine nematodes to resist oxygen stress (Modig and Olafsson, 1998; Wetzel et al., 2001). An ecosystem like OMZs with permanent hypoxia/anoxia has a potential for developing a nematode community dominated by tolerant genera with high density and consequently, decreasing relative densities of all other species (Guilini et al., 2012; Singh and Ingole, 2016). The presence of some nematode species in the OMZs may indicate that a low oxygen environment (the lower limit till 1.44 µmol l^{-1}) is favorable condition for them (e.g. Neira et al., 2001; Neira and Decraemer, 2009; Urkmez et al., 2015; Singh and Ingole, 2016). However, the low nematode diversity in the OMZs in comparison with the outside of the zone might speculate that OMZs have isolated hypoxia-tolerant nematode species as a result of their evolutionary history (Wetzel et al., 2001) and their different life strategies, small size, lower mobility, and lack of pelagic larval stage (Singh and Ingole, 2016). In such environment, other factors like food quality (Cook et al., 2000; Neira et al., 2001) or sediment Chl *a* content (Levin et al., 1991) become important to shape the nematode community. In contrast, coastal waters continuously oxygenated due to the oxygen exchanges across the air-sea surface and oxygen produced by phytoplankton. Here, oxygen availibility is a factor controlling nematode community composition (e.g. Wetzel et al., 2001; Steyaert et al., 2005; Vanaverbeke et al., 2004, 2011) and the responses of coastal nematode communities to oxygen depletion is related to local community

composition as well as to the duration of the oxygen stress. In general nematode communities are less affected by short-term oxygen depletion (less than one week; Jensen, 1984; Arroyo et al., 2012, Taheri et al., 2014, 2015) while longer term exposure leads to decreased density and diversity (Travizi, 1998, 2000; Steyaert et al., 2007; Gambi et al., 2009; Van Colen et al., 2009).

The effect of longer-term hypoxia and anoxia on macrofauna communities is more severe than short-term oxygen stress (Stachowitsch, 1984, Van Colen et al., 2008). For example, over a period of two weeks of anoxia, a mass mortality in macro-epifauna and infauna community was observed in the Gulf of Trieste where the surface areas affected by anoxiac was estimated to cover several hundred km^2. Mortality starts after 2–3 days and several macrofaunal groups, including burrowing shrimp, echinoids, polychaetes, sipunculids and bivalves appeared on the sediment surface. Within one week, sea stars and all ophiurids and hermit crabs had died (Stachowitsch, 1984). The effect of a longer period of experimentally induced hypoxia (40 days in 16 m^{-2}) on intertidal macrofauna communities was studied at Paulinapolder, a tidal flat located along the southern shore of the polyhaline part of the Westerschelde estuary, The Netherlands. The macrofaunal communities at this site consists of 18 species (~30 000 ind. m^{-2}) and is numerically dominated by polychaetes, oligochaetes and bivalves. At the end of the experiment (40 days), the results showed a complete mortality of the whole macrobenthic community (Van Colen et al., 2008). Both mentioned studies confirm the sensitivity of macrofauna to oxygen stress. In contrast to macrofauna, marine nematodes (e.g. Taheri et al., 2015) and Foraminifera (e.g. Langlet et al., 2013) are more tolerant to longer-term oxygen

stress and therefore, can have more prominent role in biogeochemical cycles in benthic ecosystems affected by oxygen stress.

Marine nematodes can stimulate carbon and nitrogen mineralization in marine sediments (e.g. Rysgaard, 2000). Denitrification is an anaerobic respiration pathway that uses nitrate as an electron acceptor. This process can reduce the effects of excessive nitrogen availability in aquatic ecosystems subject to eutrophication (Bonaglia et al., 2014). In anoxic sediment organic matter is mineralized (i.e denitrification) mainly by bacteria (Jørgensen, 1982; Plugge et al., 2011). Macrofauna are generally known to increase denitrification due to their bioturbation and bioirrigation activities (Van Colen et al., 2012; Stief, 2013; Yazdani Foshtomi et al., 2015). They are susceptible to anoxic condition and mass mortality can occur when subjected to anoxia (Stachowitsch, 1984; Stachowitsch et al., 2007; Sturdivant et al., 2013). It is shown that meiofauna bioturbation (not nematodes alone) can increase denitrification in sediments when macrofauna abundance is reduced or absent (Bonaglia et al., 2014). However, the capacity of nematodes for stimulation of denitrification is probably species-specific (Hentschel et al., 1999; Bonaglia et al., 2014). These two papers showed a contrasting result suggesting further studies are necessary to investigate the effect of nematodes on denitrification in anoxic sediments.

In conclusion, long-term anoxia can influence on functional aspect and community characteristics of the coastal nematode community. Our results showed a species-specific response of marine nematode community to anoxia which was related to exposure duration but, did not show any exact/specific adaptation mechanisms of survived nematodes to long-term anoxia. Total and vertical nematode density, species

richness and diversity decreased at 23 days and decreased further at 307 days anoxia. Surviving nematode species at long-term anoxia (307 days) may be used as indicators of oxygen stress.

5.3. The recovery patterns of nematode community

The recovery of ecosystems already affected by hypoxia/anoxia depends on the severity, duration and extension (area) of the oxygen stress. Generally it starts when the dissolved oxygen concentration in the water column again reaches 3 mg l^{-1} (Steckbauer et al., 2011). In chapter 3, the potential of nematode community recovery was examined following experimentally-induced anoxia. It is necessary to note that recovery of the sediment as a habitat is not always simultaneous with the reoxygenation of the overlaying water, especially in deeper sediment layers, as some part of the newly available oxygen is used for the re-oxidation of reduced compounds that accumulate during oxygen depletion (Wetzel et al., 2002; Van Colen et al., 2012). Recovery of the sediment may be accelerated through recolonization of the local macrobentic community (Van Colen et al., 2009). Macrobenthic activities (bioturbation and bio-irrigation) transport food and oxygen from the surface sediment to deeper layers, creating a suitable habitat for smaller infauna like nematodes (Braeckman et al., 2011). On the other hand, recolonization is the first step in the recovery process. Recolonization of nematode communities can occur through different pathways: migration through the sediment (Schratzberger et al., 2004), or through passive transport by water currents (Vieira and Fonseca, 2013), rather than by reproduction of surviving nematodes (Guerrini et al., 1998; Wetzel et al., 2001). A successful recovery

is finished when the nematode community structure and function return to the pre-anoxic conditions.

In our study, recovery started when the experimental chambers were removed (chapter 3). During the recolonization phase of 30 days after a 23 days period of anoxia, nematode species richness increased and epistrate feeding nematodes (*Spilophorella euxina*) reappeared in the dominant community. This could be related to increasing diatom concentration in the sediment due to their settlement from the water column or arriving by currents after removing the chamber. In contrast, the total density, Shannon diversity and evenness after 30 days did not fully resemble the values from the Normoxia treatments. With regard to the fact that the highest H_2S concentration and organic matter content (decaying macrofauna and other meiofauna) were observed in 23 days anoxia treatment (Metzger et al., 2014), there was probably a lag between sediment reoxygenation and a full recovery of sediment biogeochemistry (sediment biogeochemistry was not investigated after sediment reoxygenation). On the other hand, lack of planktonic life stages, low fecundity of surviving or newly arrived nematodes can hamper an increase in density during 30 days recovery (Platt and Warwick, 1988). Finally, competition among nematodes for the habitat and food as well as predator pressure by larger organisms (Blasnig et al., 2013) could result in overall lower density. Therefore, after 30 days of reoxygenation, nematode community was still in the recolonization phase and a full recovery needs more time. In contrast, the nematode community exposed to 307 days of anoxia recovered completely (density, species richness, diversity and feeding type distribution) after 90 days of reoxygenation. This could be related to both the recovery of the sediment biogeochemistry as well as to

the short life cycles (20–30 days) in most marine nematodes with a continuous reproduction (Platt and Warwick, 1988). However, the nematode community at the recovery phase was influenced by the community from the surrounding area (dominant at Normoxic cores) and except (*Actinonema pachydermatum*) all the other dominant nematodes in recovery phases (30 and 90 days) belong to the C–P class 2 and 3 (Bongers et al., 1991) which are intermediate between colonisers and persisters (Pape et al., 2013).

Most marine macofaunal organisems are mobile and have pelagic larvae; therefore the recovery mechanisms in this group are different from the pattens observed for nematodes (no planktonic stage). In the Northern Adriatic Sea (Gulf of Trieste, the same station as chapter 3), Blasnig et al. (2013) showed that hermit crabs (*Paguristes eremite*, after 30 min) and gastropods (*Hexaplex trunculus* after 3 h) were the first macrofauna animals arrived quickly in the impacted area (0.25 m^{-2}). There is no data about the macroinfauna recolonisation and recovery as the result of this study was based on the use of a time-lapse camera (Blasnig et al., 2013). However, this result highlights the role of active movement in the recovery process. In another study, macrofauna recolonisation patterns after complete defaunation (16 m^{-2}) by experimentally induced hypoxia were investigated in a polyhaline, estuarine mudflat. This paper clearly showed that colonizing assemblages were predominantly determined by juvenile recruitment (after 28 days recovery), and highlights the role of pelagic larval stage in recruitment and recolonization of macrobenthic fauna (Van Colen et al., 2008; 2010b).

It is necessary to note that the recovery process observed in this thesis is related to a small scale (0.25 m^{-2}). Patterns at larger scales tend to be more complex. For recolonization and recovery processes, the ratio between the edge and the surface of impacted area is important. In a small-scale study this ratio is greater than fore a larger scale disturbance, therefore, nematode colonization through adult migration from surrounding unimpacted areas may have facilitated recovery (Whitlatch et al., 1998). This is not taking place in the central parts of larger disturbed areas. Lack of planktonic life stages in marine nematodes (Platt and Warwick, 1988) prevent the fast recolonization through the water column, which is entirely reliant upon nematode settlement through passive transport (Vieira and Fonseca, 2013) which is in turn related to the local hydrodynamics. In an area like the north Adriatic Sea with long water residence time (250 days) (Poulain and Hariri, 2013) complete recovery at the community level at larger scales probably required a longer period. In addition, some other factors including food availability, competition for space and food, predator-prey interactions and macrofaunal recovery dynamics are also important. More studies, especially in natural ecosystems where anoxia was observed, from the center of impacted areas till the different edges, are needed to understand this process at the larger scale.

5.4. Conclusion

The results of the present thesis increased our knowledge on the effect of oxygen stress on coastal nematode communities from different sediment types. We showed that short-term oxygen stress did not have negative effects on nematode community characteristics and their feeding activity. In longer-term anoxic conditions, a decline in density and change in nematode community composition as well as functional attributes have been observed. However, some nematode species survived even 307 days anoxia. Although an increase in species richness (recolonization) was observed after 30 days, reoxygenation (sediment exposed to 23 days anoxia), total density, Shannon diversity and evenness did not fully recover. In contrast, after 90 days of reoxygenation (sediment exposed to 307 days anoxia) full recovery of total and vertical density, species richness and diversity indices as well as feeding type contribution were observed. Finally, a comparison of the effect of hypoxia/anoxia on the meiofaunal and macrofaunal communities revealed a higher resistance of meiofauna (nematodes and Foraminifera in particular) to the short and long-term oxygen stress (Riedel et al., 2012; Langlet et al., 2013; Taheri et al., 2015).

5.5. Future challenges

Although information on the effect of oxygen stress on nematode community characteristics has gradually increased, the effect of oxygen stress on the physiological and reproductional aspects of nematodes are still not well understood.

In marine sediments, benthic microbial communities shift to sulfate reduction as hypoxia/anoxia progresses, and sulfide concentrations increase in the environment (Conley et al., 2009). During hypoxic events, the anoxic layer of the sediments migrates

upwards and can reach the water column, with sulfide intrusion into the bottom water (Metzger et al., 2014). As hydrogen sulphide is toxic for marine benthic animals (Diaz and Rosenberg, 1995), the negative effects of oxygen stress on macrofaunal biodiversity can be aggravated in the presence of sulphide (Vaquer-Sunyer and Duarte, 2010). Therefore, a laboratory study on the effect of different levels of hydrogen sulphide concentration, and exposure time on the nematode community can be recommended.

The decrease in respiration rate (Ott and Schiemer, 1973) which has been observed in some marine nematodes in prolonged anoxia could be related to a reduction in their activity and a switch to an anaerobic metabolism (Wieser and Kanwishe, 1961). Anaerobic metabolism is not as efficient as aerobic metabolism and only little amounts of energy (12-13 percent of total achievable energy in aerobic metabolism) are yielded by this process (Megonigal et al., 2004). The low energy yield from the available food can hamper nematode growth and lead to unsuccessful reproduction and survival of juvenile nematodes (Austen and Wibdom, 1991). Reproduction is a high-energy activity and would require oxygen. If nematodes decrease their feeding activity or shift to anaerobic metabolism, the energy yield could be too low to support reproduction. One the other hand, a reduction in the activity can disturb (Wieser and Kanwishe, 1961) nematode dispersal since they don't have planktonic life stages. If nematodes remain in their normal rate of activity in anoxic condition, they have to consume more to obtain enough energy for growth and reproduction. In our feeding experiment (chapter 4), no negative effect of short-term hypoxia on the nematode feeding activity was observed. The same behaviour could also be seen during longer term exposure. Feeding activity

of nematode community under anoxia is not fully understood yet. Therefore, a laboratory study on the effect of different durations of anoxic circumstances on the feeding activity (carbon stable isotope analysis) of the nematode community (like chapter 4), especially focusing on those species surviving long-term anoxia is suggested for future studies. The results can provide important insights in to the feeding activity and adaptation of organisms under anoxia.

This thesis mainly focuses on the effect of oxygen stress on nematode community characteristics. Jensen (1995) provided some evidence that the tolerance range of the adults and juveniles of the nematode species *Theristus anoxybioticus* to anoxia is different. Reproducing adults of this species need to be in the oxygenated sediment layers even for a short time, while juveniles were typically limited to the deeper anoxic layers, suggesting that oxygen is an essential requirement for reproduction. In addition, the duration of the juvenile live stages under anoxia was about four to eight times longer than for other species with similar body size living in oxic conditions (Jensen, 1995). Therefore it can be hypothesized that oxygen stress affects reproduction and larval development of nematodes. As reproduction is a high-energy demanding activity requiring oxygen (Jensen, 1995), it is possible that nematode species postpone their reproduction in anoxic condition to save energy and avoid mortality. For future research, a laboratory experiment can be recommended to study the life cycle (juvenile/adult ratio, number of eggs, minimum generation time and life span) of tolerant nematodes to long-term oxygen stress under different duration of anoxia as described by Zaika and Makarova (1979) and Van Campenhout et al. (2014).

As previously mentioned, nitrate respiration has been observed in some Foraminifera (Bernhard et al., 2012; Nomaki et al., 2015) and two marine nematode species (Hentschel et al., 1999) to adapt to oxygen-depleted environments. It is possible that the same adaptation mechanism (metabolism of nitrogen by ectosymbiotic bacteria) is more common in marine nematodes. If nitrate respiration is indeed widespread in marine nematodes, it could explain the tolerance of nematodes to long-term anoxia. Hentschel et al. (1999) incubated anaerobically nematodes in the presence of nitrate and measured both nitrate and nitrite concentration in water. Therefore, it can be recommended to study the ability of nitrate respiration in nematodes tolerant to long-term anoxia as described by Hentschel et al. (1999).

Addenda

Addendum 1. Appendices to chapter 2

Effect of short-term hypoxia on marine nematode community structure and vertical distribution pattern in three different sediment types of the North Sea

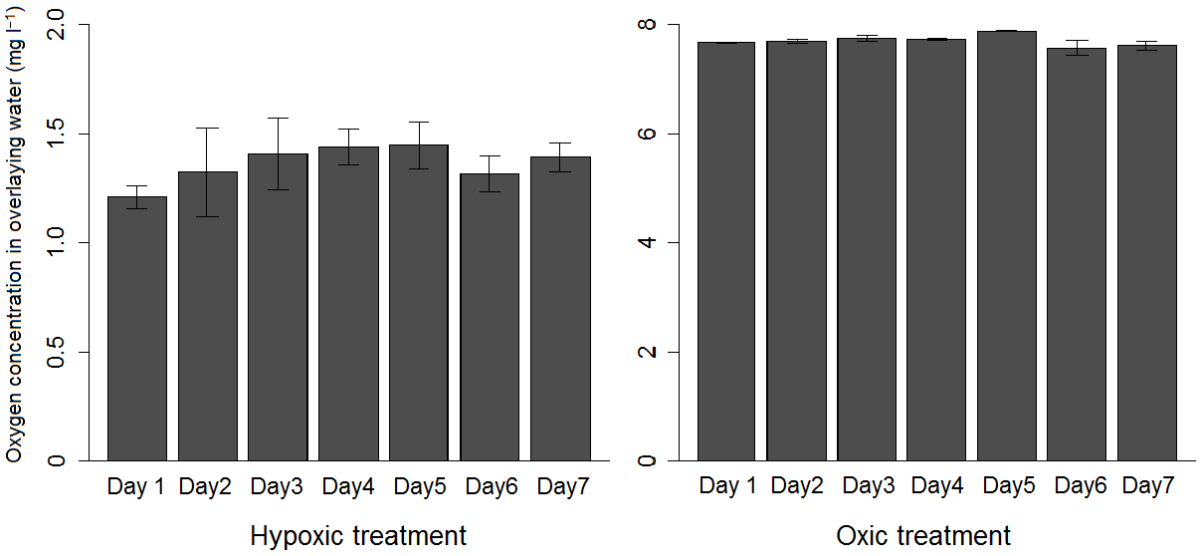

Figure S1: Oxygen concentration in overlaying water (mg l^{-1}) during the study period.

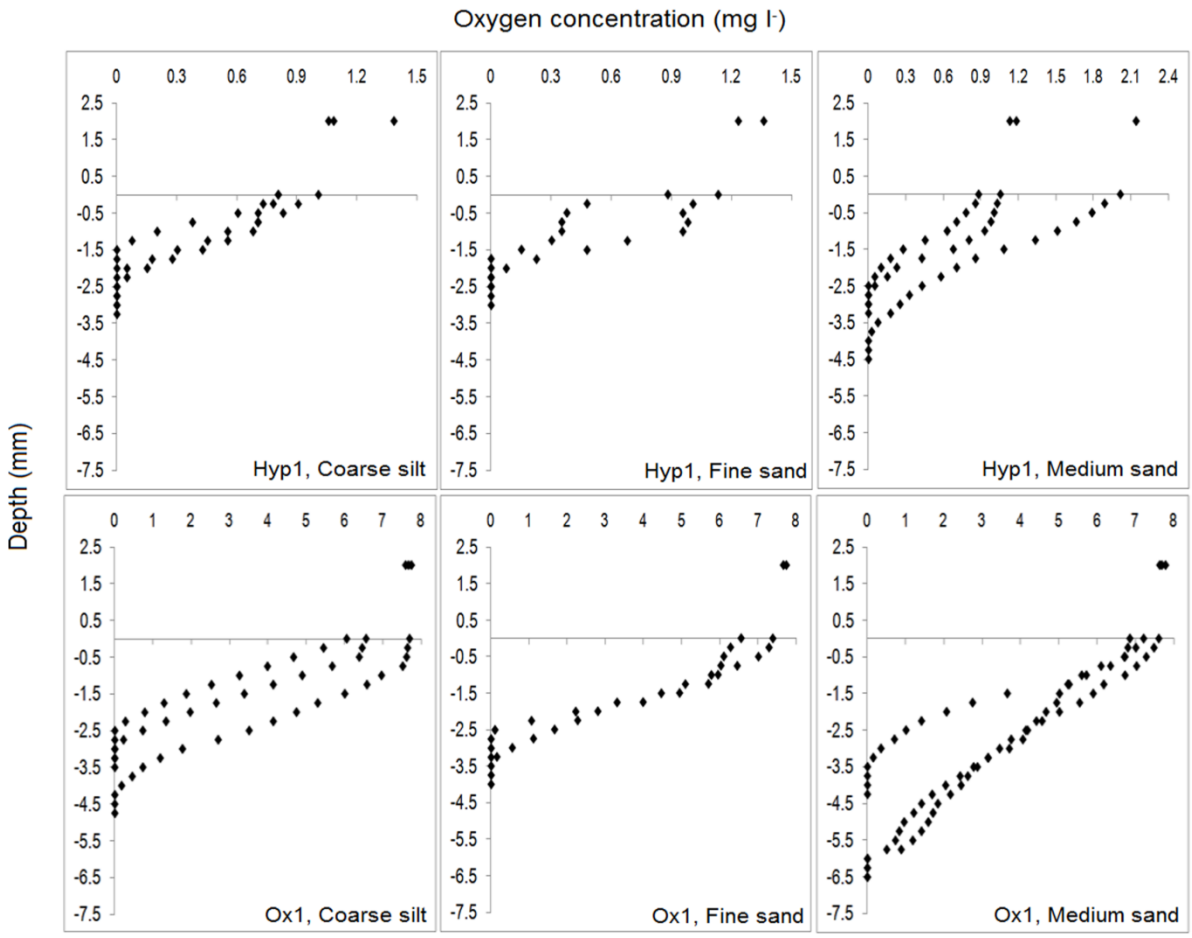

Figure S2: Oxygen profile in overlaying water and sediment (mg l^{-1}) in day 1.

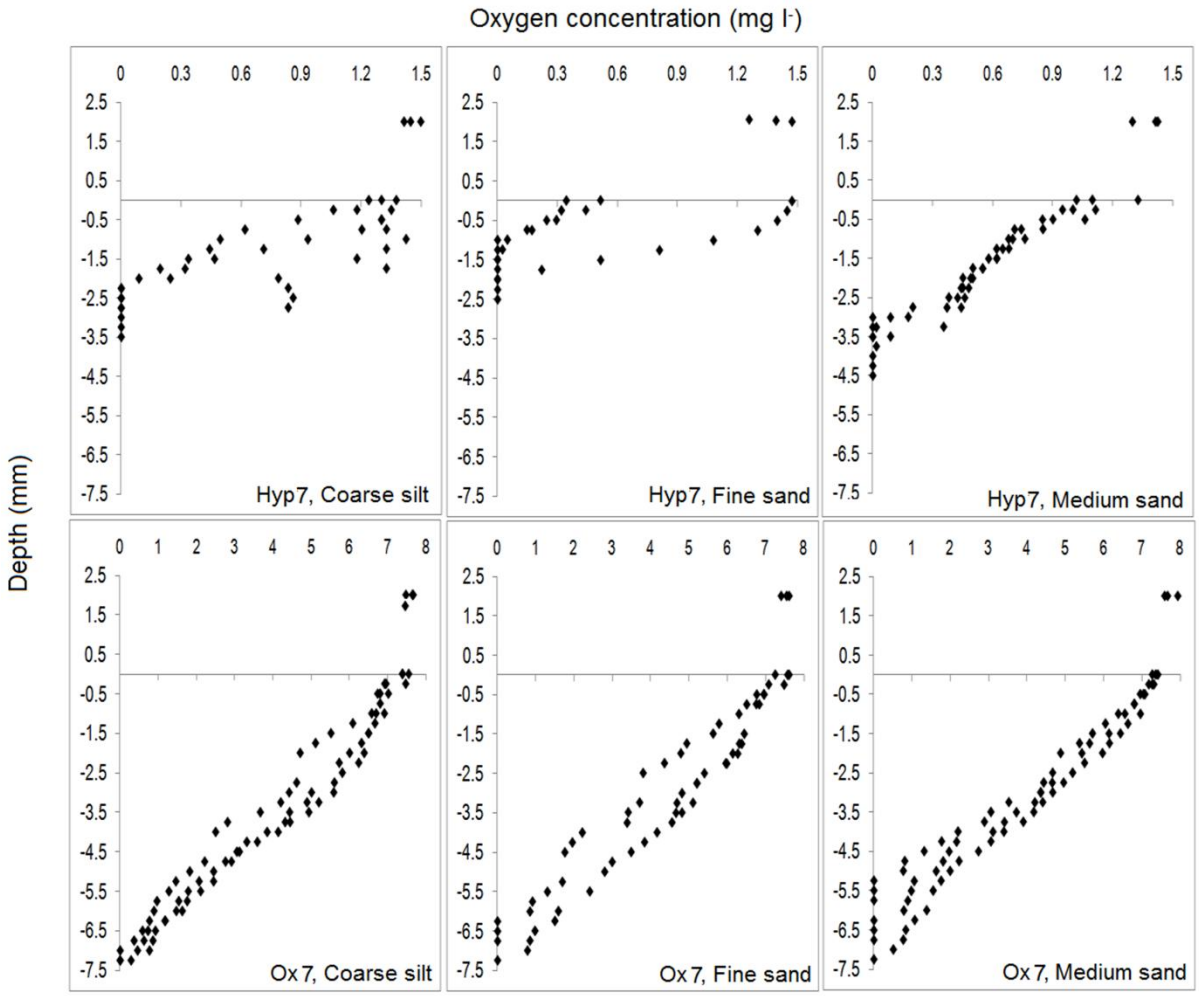

Figure S3: Oxygen profile in overlaying water and sediment (mg l^{-1}) in day 7.

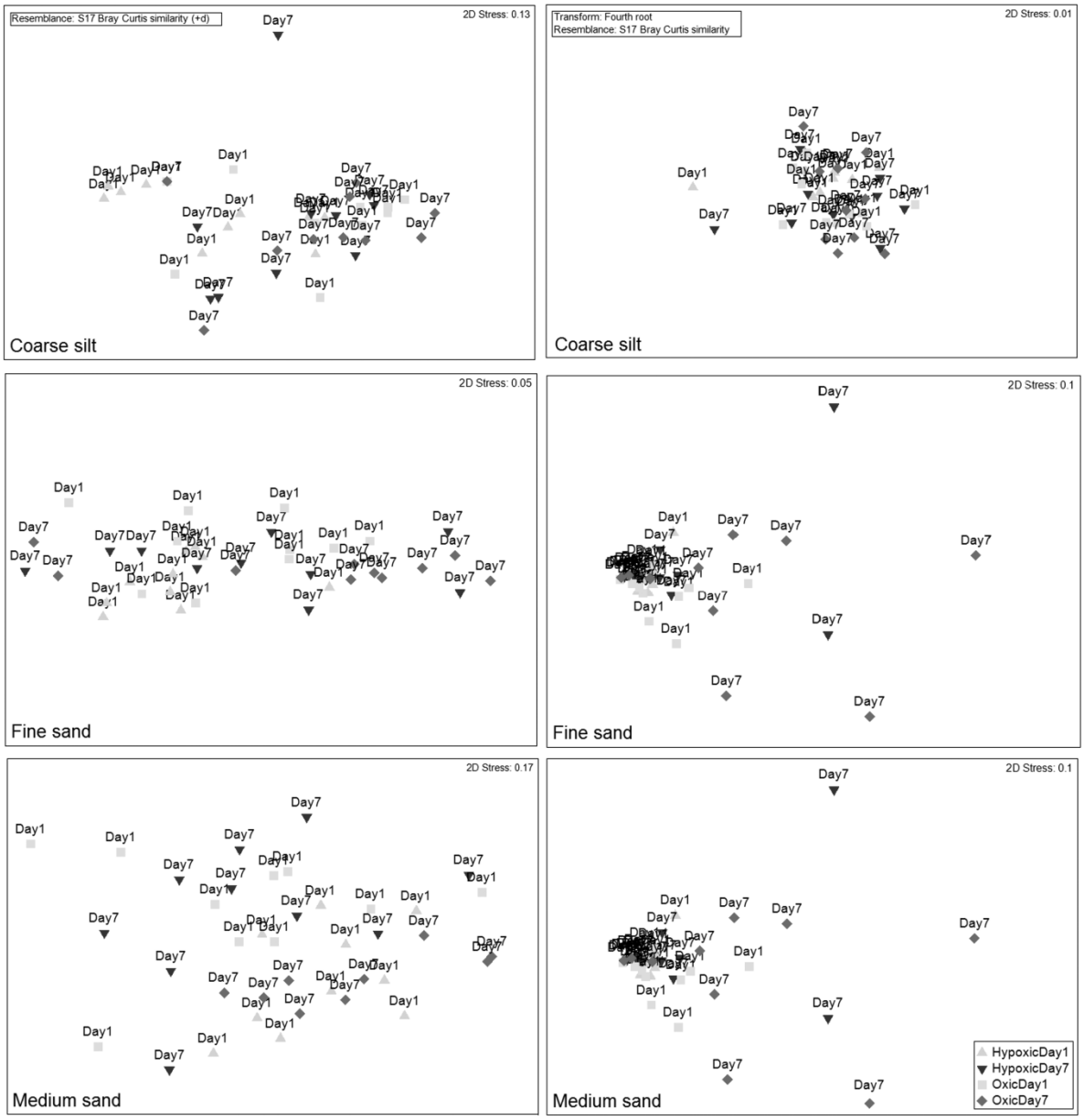

Figure S4: MDS plot of Bray-Curtis similarities based on untransformed (left) and 4th root transformed (right) nematode abundance data.

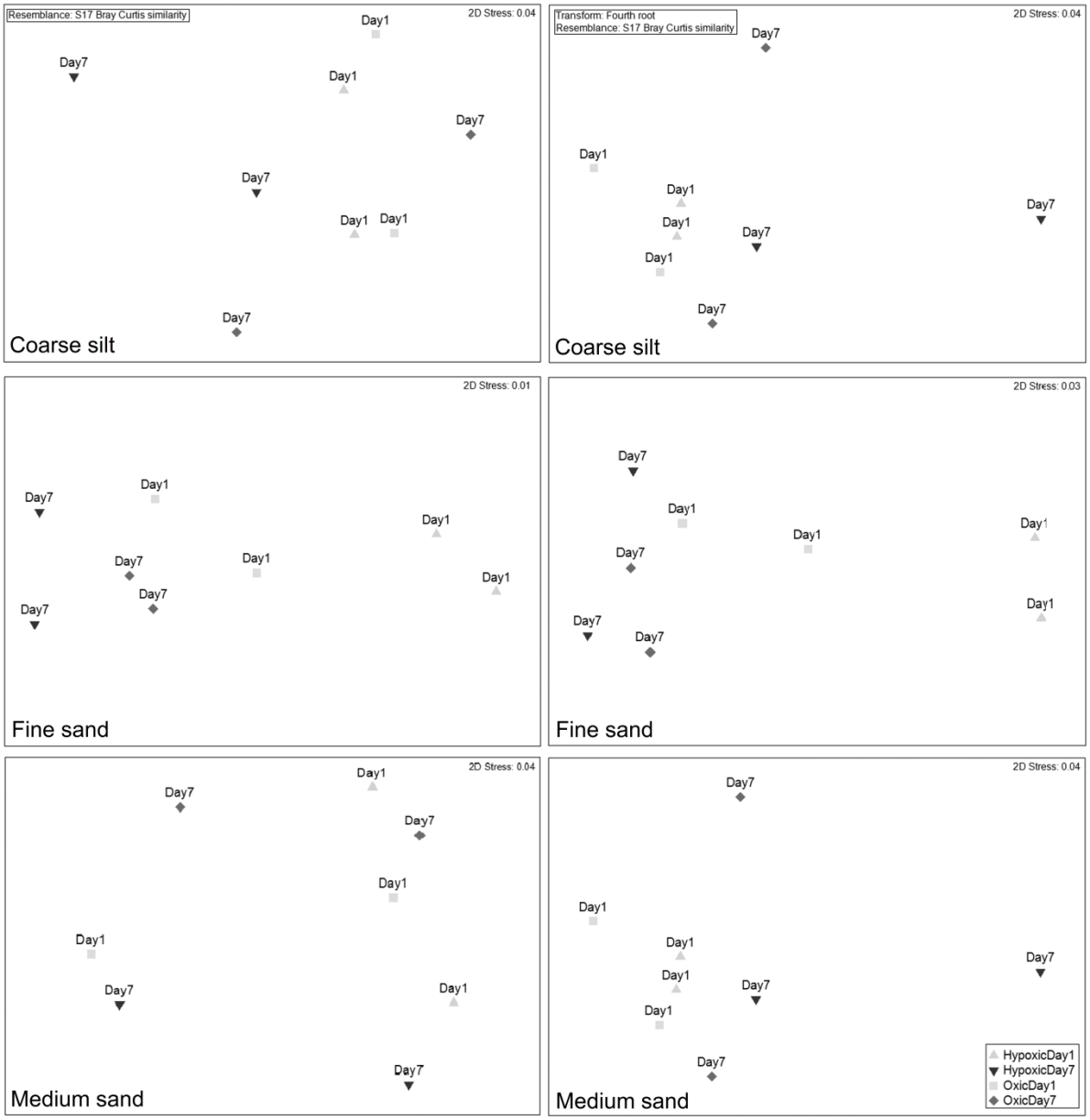

Figure S5: MDS plot of Bray-Curtis similarities based on untransformed (left) and 4th root transformed (right) nematode abundance data from 0–1 cm layer.

Table S1. Main test results from PERMANOVA analysis for differences in nematode community structure between field control and Ox1 in all stations based on 4th root transformed data. P (Per) = permutation, "*" P-values obtained from Monte-Carlo test.

	Community structure, Fc-Ox1			
	df	MS	Pseudo-F	P (Per)
Coarse silt				
Treatment	1	2762.50	0.70	1*
Slice	4	3170.50	1.04	0.435
Re(Tr)	2	3932.50	1.29	0.284
Treatment × Slice	4	2901.60	0.95	**0.552**
Fine sand				
Treatment	1	2546.10	1.14	0.342*
Slice	4	1325.00	1.06	0.412
Re(Tr)	2	2226.10	1.78	0.060
Treatment × Slice	4	1526.20	1.22	**0.253**
Medium sand				
Treatment	1	7507.20	2.33	0.331*
Slice	4	2049.00	1.30	0.137
Re(Tr)	2	3228.40	2.03	0.012
Treatment × Slice	4	1785.70	1.13	**0.311**

Table S2. Main test results from PERMANOVA analysis for differences in nematode community structure among treatment in all stations based on 4th root transformed data. P (Per) = permutation, "*" P-values obtained from Monte-Carlo test.

	Community structure, treatments			
	df	MS	Pseudo-F	P (Per)
Coarse silt				
Treatment	1	4207.00	0.95	0.664*
Day	1	5619.70	1.54	0.273
Slice	4	6205.60	2.30	0.011
Re(Tr)	2	4444.70	2.27	0.025
Treatment × Day	1	1981.10	0.54	0.670
Treatment × Slice	4	2339.40	0.86	0.667
Day × Slice	4	2523.90	1.29	0.215
Day × Re(Tr)	2	3653.80	1.87	0.064
Slice × Re(Tr)	8	2708.30	1.39	0.089
Treatment × Day × Slice	4	1858.50	0.95	**0.543**
Fine sand				
Treatment	1	1925.50	0.84	0.665*
Day	1	3155.20	1.99	0.243
Slice	4	1839.40	2.03	0.017
Re(Tr)	2	2299.50	2.80	0.010
Treatment × Day	1	906.16	0.57	0.640
Treatment × Slice	4	755.53	0.83	0.762
Day × Slice	4	933.00	1.14	0.320
Day × Re(Tr)	2	1578.60	1.92	0.045
Slice × Re(Tr)	8	905.80	1.10	0.312
Treatment × Day × Slice	4	1091.20	1.33	**0.166**
Medium sand				
Treatment	1	2258.50	0.88	0.665*
Day	1	4053.50	1.48	0.310
Slice	4	1928.10	0.94	0.564
Re(Tr)	2	2574.10	1.82	0.067
Treatment × Day	1	6157.80	2.25	0.180
Treatment × Slice	4	1860.70	0.91	0.610
Day × Slice	4	1671.90	1.18	0.283
Day × Re(Tr)	2	2751.70	1.94	0.055
Slice × Re(Tr)	8	2046.70	1.44	0.047
Treatment × Day × Slice	4	1809.00	1.28	**0.212**

Table S3. Main test results from PERMANOVA analysis for differences in nematode community structure from 0–1 cm layer between field control and Ox1 in all stations based on untransformed and 4th root transformed data. P (Per) = permutation, "*" P-values obtained from Monte-Carlo test.

		Community structure 0-1 cm Fc-Ox1, untransformed data				**Community structure 0-1 cm Fc-Ox1, 4th transformed data**		
	df	MS	Pseudo-F	P (MC)	df	MS	Pseudo-F	P (MC)
Coarse silt								
Treatment	1	5225.40	1.09	**0.410**	1	6109.00	1.57	**0.297**
Fine sand								
Treatment	1	3348.50	1.09	**0.428**	1	2095.90	1.42	**0.332**
Medium sand								
Treatment	1	3095.70	1.29	**0.357**	1	1578.90	0.84	**0.517**

Table S4. Main test results from PERMANOVA analysis for differences in nematode community structure from 0–1 cm among treatments in all stations based on untransformed and 4th root transformed data. P (Per) = permutation, "*" P-values obtained from Monte-Carlo test.

		Community structure 0-1 cm Treatment, untransformed data				**Community structure 0-1 cm Treatment, 4th transformed data**		
	df	MS	Pseudo-F	P (Per)	df	MS	Pseudo-F	P (Per)
Coarse silt								
Treatment	1	3462.30	0.77	0.730	1	2496.70	0.79	0.641
Day	1	5597.70	1.25	0.277	1	4282.30	1.36	0.246
Treatment × Day	1	2926.80	0.65	**0.803**	1	2219.20	0.70	**0.677**
Fine sand								
Treatment	1	6257.40	4.84	0.027	1	1553.50	1.73	0.077
Day	1	6649.30	5.14	0.024	1	3464.10	3.85	0.032
Treatment × Day	1	3710.30	2.87	**0.053**	1	1768.10	1.97	**0.065**
Medium sand								
Treatment	1	2158.80	0.85	0.636	1	1793.00	1.22	0.330
Day	1	1545.10	0.61	0.827	1	1507.60	1.03	0.439
Treatment × Day	1	2481.10	0.97	**0.590**	1	1787.00	1.22	**0.359**

Addendum 2. Appendices to chapter 3

Patterns in nematode community during and after experimentally induced anoxia in the northern Adriatic Sea

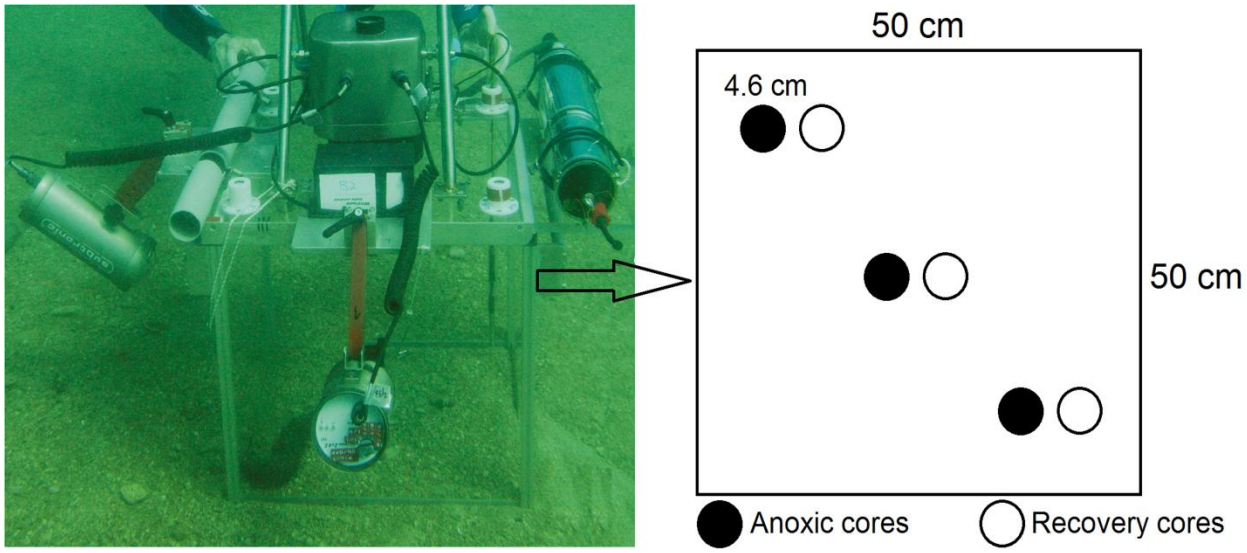

Figure S1. Schematic drawing of sampling positions in all anoxia and recovery treatments (Table 1). Black colors indicate anoxia cores and white colors indicate recovery cores under each chamber. The left figure is the Experimental Anoxia Generating Unit (EAGU) which was used in the first chamber.

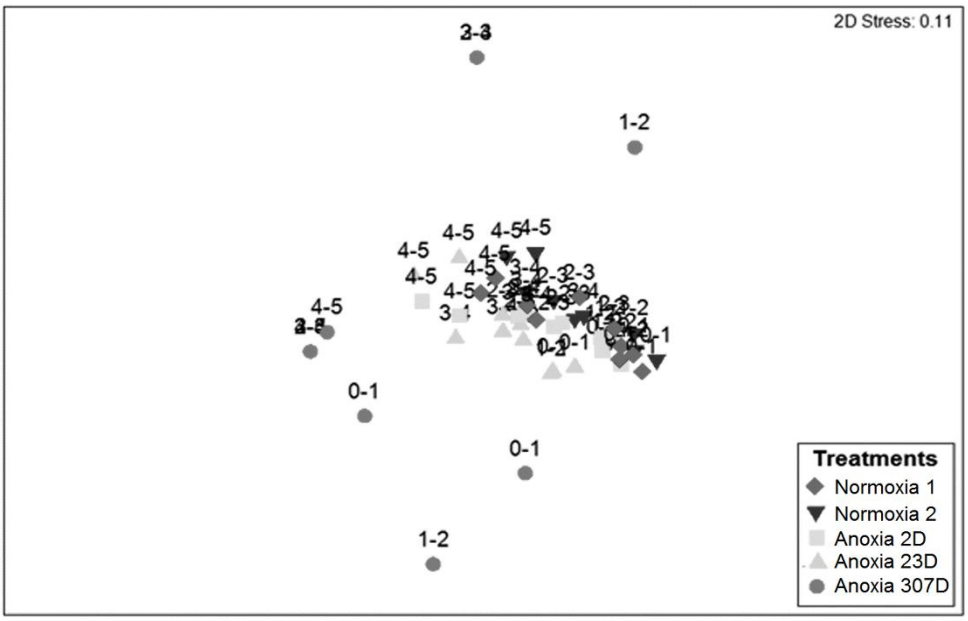

Figure S2. MDS plot based on Bray-Curtis similarity and 4th root transformed nematode density datain the full sediment column (0-5 cm) and upper sediment layers (0-2 cm) at all treatments.

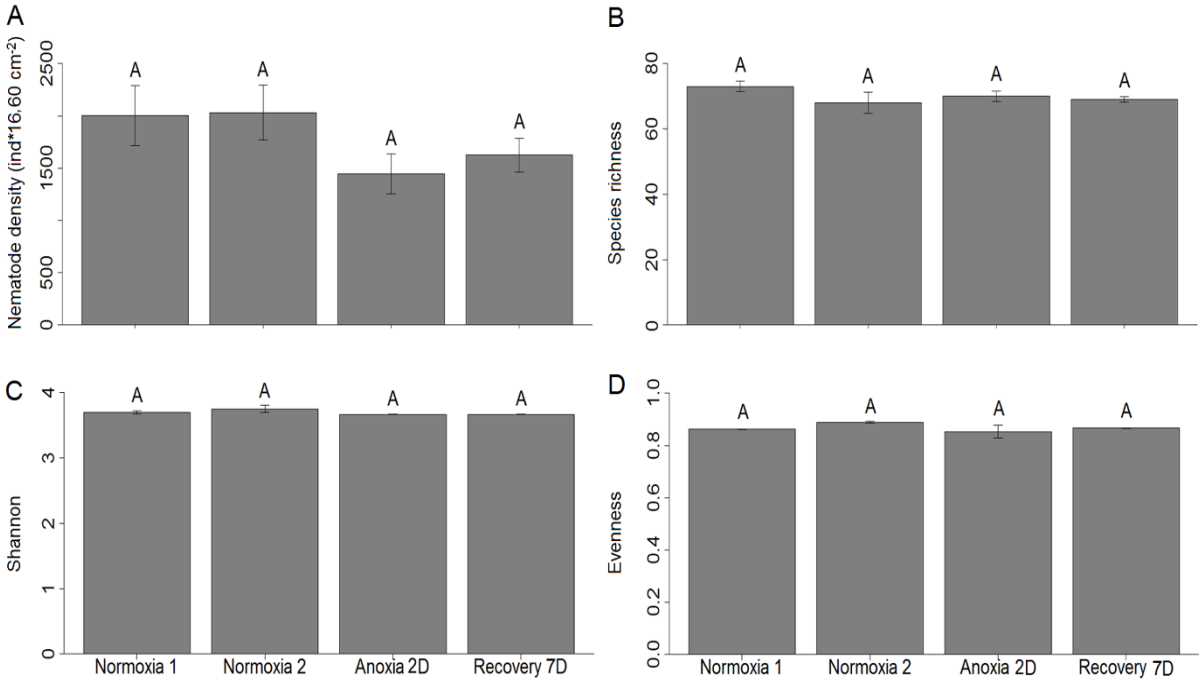

Figure S3.Total nematode densities (A) (mean±SE, n=3), Shannon diversity (B), species richness (C) and evenness (D) after 7 days recovery (mean±SE, n=2). Same capital letters indicate no significant differences ($p > 0.05$).

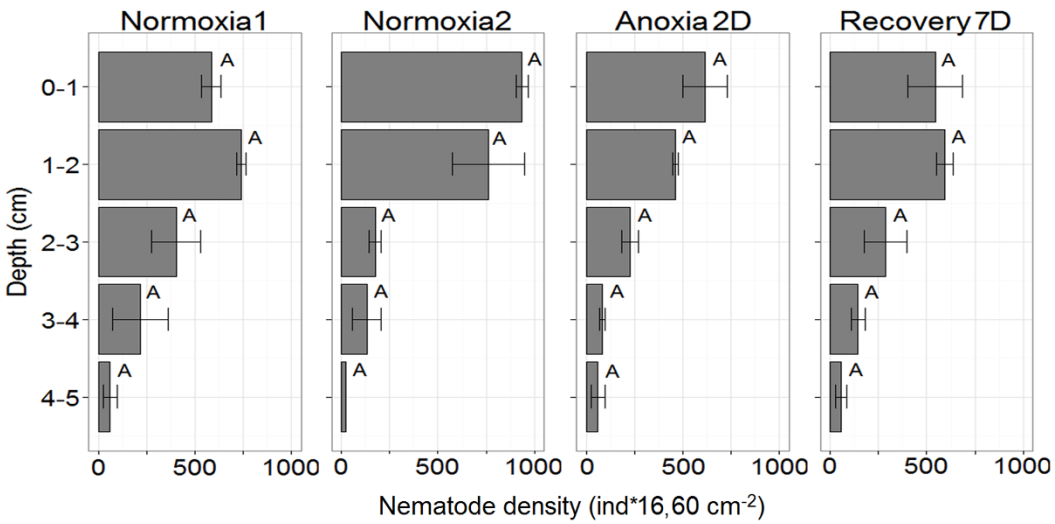

Figure S4. Vertical density profiles (mean±SE, n=3) after 7 days recovery. Same capital letters indicate no significant differences ($p > 0.05$).

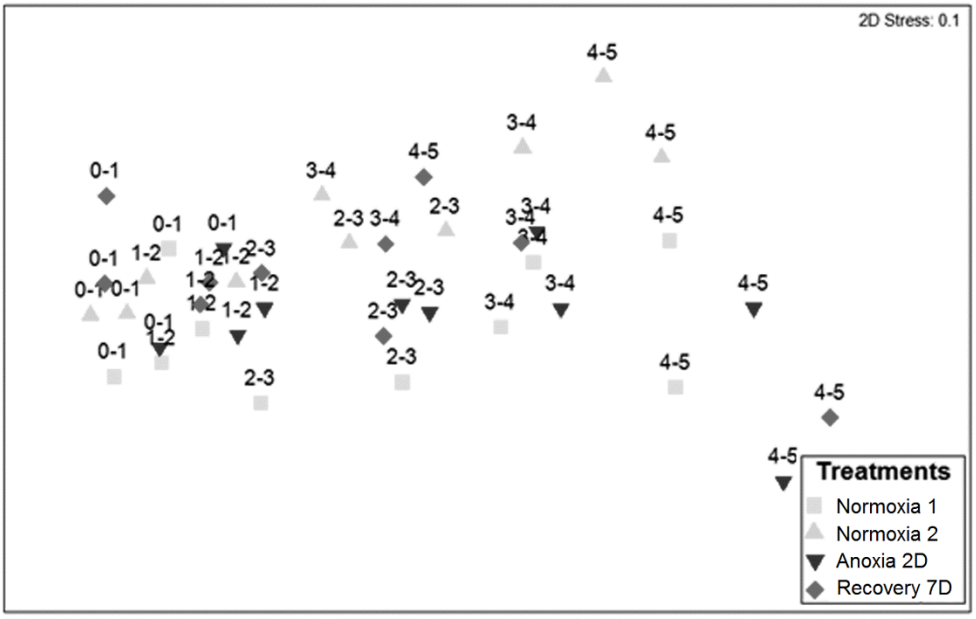

Figure S5. MDS plot based on Bray-Curtis similarity and 4th root transformed nematode density data after 7 days recovery.

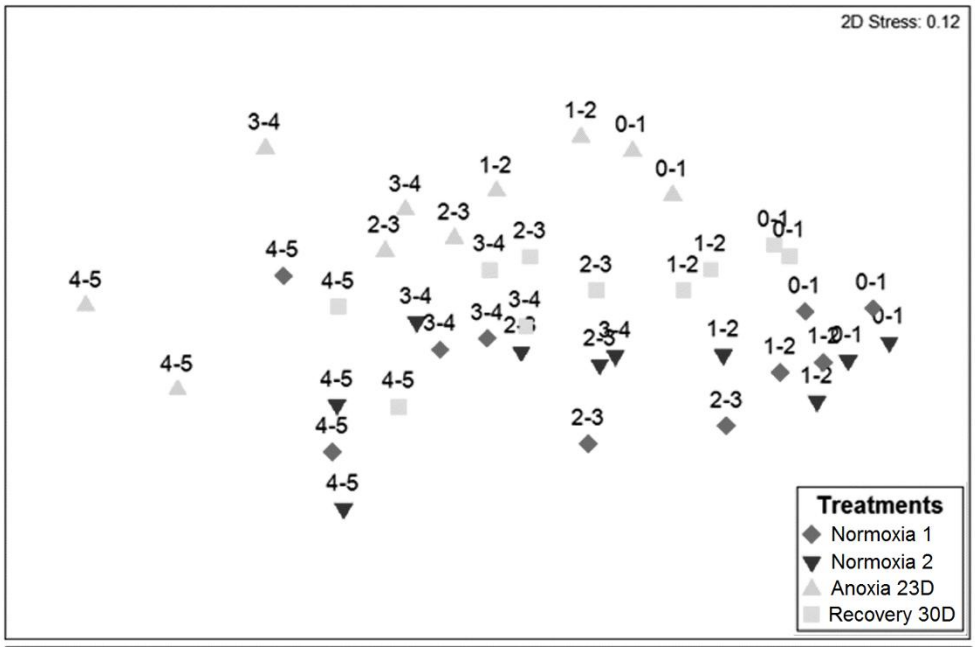

Figure S6. MDS plot based on Bray-Curtis similarity and 4th root transformed nematode density data after 30 days recovery.

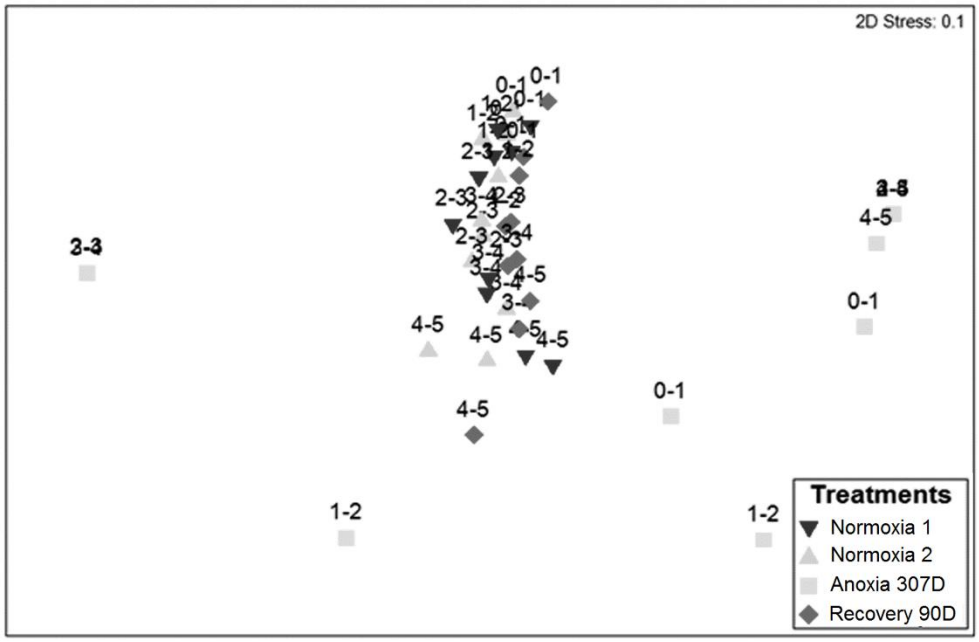

Figure S7. MDS plot based on Bray-Curtis similarity and 4th root transformed nematode density data after 90 days recovery.

Table S1. Results from one-way ANOVA and Tukey HSD test of total nematode density.

Main test	0-5 cm			
	df	ms	F	P
Treatments	4	2075905.00	18.10	0.000
Residuals	8	114684.00		

Pairwise test	
Treatments	P
Normoxia1-Normoxia 2	0.999
Normoxia1-Anoxia 9D	0.340
Normoxia1-Anoxia 1M	0.002
Normoxia1- Anoxia 10M	0.001
Normoxia2-Anoxia 9D	0.395
Normoxia2-Anoxia 1M	0.004
Normoxia2- Anoxia 10M	0.002
Anoxia 9D- Anoxia 1M	0.029
Anoxia 9D- Anoxia 10M	0.010
Anoxia 1M- Anoxia 10M	0.766

Table S2. Main and pairwise test results from PERMANOVA analysis for differences inspecies richness, Shannon and evenness.

Main test	0-5 cm			
Species richness	df	MS	Pseudo-F	P(perm)
Treatments	4	1293.40	58.52	0.009
Residuals	5	22.10		
Shannon				
Treatments	4	0.85	5.94	0.012
Residuals	5	0.14		
Evenness				
Treatments	4	0.00	0.94	0.527
Residuals	5	0.00		

Pairwise test	0-5 cm	
	Species richness	**Shannon**

Treatments	P (MC)	P (MC)
Normoxia1-Normoxia 2	0.381	0.663
Normoxia1-Anoxia 9D	0.397	0.849
Normoxia1-Anoxia 1M	0.021	0.000
Normoxia1- Anoxia 10M	0.009	0.124
Normoxia2-Anoxia 9D	0.700	0.695
Normoxia2-Anoxia 1M	0.065	0.026
Normoxia2- Anoxia 10M	0.012	0.114
Anoxia 9D- Anoxia 1M	0.026	0.082
Anoxia 9D- Anoxia 10M	0.009	0.133
Anoxia 1M- Anoxia 10M	0.018	0.233

Table S3. Main test results from two-way PERMANOVA analysis for differences in nematode vertical density, community structure, species richness, Shannon diversity and evenness among anoxia treatments. P (Per) = permutation.

		Nematode densities				**Community structure**		
	df	MS	Pseudo-F	P (Per)	df	MS	Pseudo-F	P (Per)
Treatment	4	34.64	60.16	0.000	4	10605.00	3.72	0.001
Slice	4	13.36	74.20	0.000	4	8971.40	6.11	0.000
Re(Tr)	8	0.57	3.19	0.007	5	2849.40	1.99	0.000
Treatment × Slice	16	0.45	2.52	0.014	16	2761.70	1.88	0.000
					Pair wise test		t	P (MC)
					Normox 2- Anox 1M, 0-1 cm		2.74	0.048
					Normox 2- Anox 10M, 4-5 cm		3.10	0.038
		Species richness				**Shannon diversity**		
Treatment	4	1465.70	42.14	0.013	4	9.57	89.95	0.007
Slice	4	939.83	64.46	0.000	4	2.00	33.30	0.000
Re(Tr)	5	34.78	2.38	0.072	5	0.10	1.76	0.160
Treatment × Slice	16	65.61	4.50	0.000	16	0.26	4.45	0.000
		Evenness						
Treatment	4	0.03	8.48	0.020				
Slice	4	0.06	6.36	0.002				
Re(Tr)	5	0.03	4.03	0.008				
Treatment × Slice	16	0.00	2.98	0.009				

Table S4. Main test results from one-way ANOVA and PERMANOVA analysis for differences in nematode total density, species richness, Shannon diversity and evenness among 2 days anoxia-recovery treatments. P (MC) = Monte Carlo. "*" P-values obtained from ANOVA test.

	full sediment columns (0-5 cm)		
	df	Pseudo-F	P (MC)
Total density	3,7	1.46	0.306*
Species richness	3,7	0.75	0.582
Shannon diversity	3,7	0.40	0.754
Evenness	3,7	1.05	0.462

Table S5. Main test results from two-way PERMANOVA analysis for differences in nematode vertical density, community structure, species richness, Shannon diversity and evenness 2 days anoxia-recovery treatments. P (Per) = permutation.

		Nematode densities				**Community structure**		
	df	MS	Pseudo-F	P (Per)	df	MS	Pseudo-F	P (Per)
Treatment	3	44720.00	1.46	0.293	3	2973.80	1.59	0.010
Slice	4	861150.00	55.27	0.000	4	11114.00	8.82	0.000
Re(Tr)	7	30683.00	1.97	0.092	4	1864.70	1.48	0.016
Treatment × Slice	12	28770.00	1.85	0.088	12	1388.20	1.10	0.213
		Species richness				**Shannon diversity**		
Treatment	3	14.49	0.32	0.817	3	0.21	1.51	0.290
Slice	4	1308.10	65.69	0.000	4	1.44	23.17	0.000
Re(Tr)	4	44.97	2.26	0.104	4	0.14	2.24	0.104
Treatment × Slice	12	24.55	1.23	0.340	12	0.11	1.75	0.142

		Evenness		
Treatment	3	0.00	1.70	0.212
Slice	4	0.00	1.53	0.241
Re(Tr)	4	0.00	3.04	0.049
Treatment × Slice	12	0.00	2.04	0.089

Table S6.Main test results from one-way ANOVA and PERMANOVA analysis for differences in nematode total density, species richness, Shannon diversity and evenness among 23 days anoxia-recovery treatments. P (MC) = Monte Carlo. "*" P-values obtained from ANOVA test.

	full sediment columns (0-5 cm)		
	df	Pseudo-F	P (MC)
Total density	3,7	14.95	0.001*
Species richness	3,7	13.31	0.015
Shannon diversity	3,7	27.49	0.004
Evenness	3,7	29.57	0.004

Table S7. Main test results from two-way PERMANOVA analysis for differences in nematode vertical density, community structure, species richness, Shannon diversity and evenness 23 days anoxia-recovery treatments. P (Per) = permutation.

		Nematode densities				**Community structure**		
	df	MS	Pseudo-F	P (Per)	df	MS	Pseudo-F	P (Per)
Treatment	3	36034.00	14.95	0.003	3	5727.60	3.30	0.009
Slice	4	48283.00	62.27	0.000	4	9181.00	8.59	0.000
Re(Tr)	7	24098.00	3.11	0.013	4	1736.30	1.62	0.005
Treatment × Slice	12	69350.00	8.94	0.000	12	1984.50	1.86	0.000
					Pair wise test		t	P (MC)

		Species richness				**Shannon diversity**		
Treatment	3	322.29	6.42	0.08	3	0.83	10.49	0.063
Slice	4	914.48	55.25	0.00	4	0.72	13.78	0.000
Re(Tr)	4	50.17	3.03	0.048	4	0.00	1.52	0.240
Treatment × Slice	12	28.87	1.74	0.153	12	0.00	0.92	0.544

		Evenness		
Treatment	3	0.02	11.49	0.018
Slice	4	0.01	8.92	0.001
Re(Tr)	4	0.00	1.42	0.279
Treatment × Slice	12	0.00	1.70	0.162

Table S8. Main test results from one-way ANOVA and PERMANOVA analysis for differences in nematode total density, species richness, Shannon diversity and evenness among 307 days anoxia-recovery treatments. P (MC) = Monte Carlo. "*" P-values obtained from ANOVA test.

	full sediment columns (0-5 cm)		
	df	Pseudo-F	P (MC)
Total density	3,6	22.11	0.001
Species richness	3,7	53.96	0.001
Shannon diversity	3,7	6.58	0.540
Evenness	3,7	0.41	0.756

Table S9. Main test results from two-way PERMANOVA analysis for differences in nematode vertical density, community structure, species richness, Shannon diversity and evenness307 days anoxia-recovery treatments. P (Per) = permutation.

		Nematode densities				**Community structure**		
	df	MS	Pseudo-F	P (Per)	df	MS	Pseudo-F	P (Per)
Treatment	3	38034.00	8.05	0.026	3	11376.00	3.64	0.010
Slice	4	42514.00	37.40	0.000	4	7197.50	4.31	0.000
Re(Tr)	6	47234.00	4.16	0.004	4	3124.70	1.87	0.003
Treatment × Slice	12	71606.00	6.31	0.000	12	2714.70	1.62	0.000

		Species richness				**Shannon diversity**		
Treatment	3	1949.50	42.03	0.124	3	13.09	342.18	0.049
Slice	4	721.91	52.50	0.000	4	1.44	24.39	0.000
Re(Tr)	4	46.37	3.37	0.034	4	0.00	0.65	0.638
Treatment × Slice	12	65.60	4.77	0.002	12	0.29	5.01	0.002

		Evenness		
Treatment	3	0.00	17.01	0.021
Slice	4	0.00	4.79	0.010
Re(Tr)	4	0.00	1.47	0.261
Treatment × Slice	12	0.00	1.00	0.485

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A1- peer reviewed articles

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Article in prepration

Taheri, M., Giunio, M., De Troch, M., Vincx, M., Vanaverbeke, J. Effect of short-term hypoxia on the feeding activity of abundant nematode genera from an intertidal mudflat.

Presentations

Taheri, M., Braeckman, U., Vincx, M., Vanaverbeke, J., 2012. Short-term hypoxia does not affect nematode densities and vertical distribution patterns at the Belgian Part of the North Sea, ***in***: Mees, J. *et al.* (Ed.) (2012). *Book of abstracts - VLIZ Young Marine Scientists' Day. Brugge, Belgium, 24 February 2012. VLIZ Special Publication,* 55: pp. 81

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